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Numerical Classification of Sharp-beaked Redfishes, *Sebastes mentella* and *S. fasciatus*, from Northeastern Grand Bank

I-H. Ni

Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, Research and Resource Services, P.O. Box 5667, St. John's, Nfld. A1C 5X1

Ni, I-H. 1981. Numerical classification of sharp-beaked redfishes, *Sebastes mentella* and *S. fasciatus*, from northeastern Grand Bank. Can. J. Fish. Aquat. Sci. 38: 873–879.

The distinction between *Sebastes fasciatus* and *S. mentella* has not previously been clearly established as good morphological discriminators had not been isolated. Eleven meristic and 14 morphological characters were examined individually (univariate statistics) and collectively (discriminant analysis) on 200 sharp-beaked redfish specimens representing both *S. fasciatus* and *S. mentella* which had been separated by the anatomical character of extrinsic gasbladder musculature. The result of the discriminant analysis demonstrated good (100%) separation of redfish with combinations of meristic and morphological characters. Anal fin rays, relative position of pectoral and pelvic fin to anus, number of the first left gill rakers, angle of the third preopercular spine, the fusion of the occipital–nuchal ridge, and vertebral number were good discriminators between *S. mentella* and *S. fasciatus*.

Key words: *Sebastes fasciatus*, *S. mentella*, redfish, taxonomy, morphology, identification, discriminant analysis

Ni, I-H. 1981. Numerical classification of sharp-beaked redfishes, *Sebastes mentella* and *S. fasciatus*, from northeastern Grand Bank. Can. J. Fish. Aquat. Sci. 38: 873–879.

L'impossibilité d'isoler de bons discriminants morphologiques avait jusqu'à maintenant empêché d'établir une claire distinction entre *Sebastes fasciatus* et *S. mentalla*. Nous avons examiné 11 caractères méristiques et 14 caractères morphologiques individuellement (statistique à une variable) et collectivement (analyse discriminante) chez 200 sébastes à museau pointu. Ce lot contenait à la fois *S. fasciatus* et *S. mentella* qui avaient été séparés par le caractère anatomique de la musculature intrinsèque de la vessie gazeuse. L'analyse discriminante démontre qu'il y a bonne séparation (100%) des sébastes à l'aide de combinaisons de caractères méristiques et morphologiques. Les rayons de la nageoire anale, la position relative des nageoires pectorales et pelviennes par rapport à l'anus, le nombre de branchicténies sur

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la première branchie gauche, l'angle de la troisième épine préoperculaire, la fusion de la crête occipito–nuchale et le nombre de vertèbres sont de bons discriminants entre *S. mentella* et *S. fasciatus*.

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A good understanding of *Sebastes* sp. composition and stock units is necessary for better stock assessment and management. One of the basic methods to approach this problem is through morphological studies. However, the classification of the Northwest Atlantic sharp-beaked redfishes has been somewhat confused in the past as all the morphological characters investigated overlapped. Although the anatomical character of extrinsic gasbladder musculature can serve as a clear-cut discriminator between *S. fasciatus* and *S. mentella* (Ni 1981), the dissection is very time-consuming which makes it difficult to apply in field studies. Therefore, the aim of this paper is to determine good morphological discriminators and to offer guidelines for separating sharp-beaked redfishes in the field.

The existence of a form of sharp-beaked redfish other than *S. mentella* was suggested by Barsukov (1968). This different form had been briefly described more than 100 yr ago on the basis of only one young specimen and so retains the original name *S. fasciatus* Storer 1856. Barsukov (1972) listed some distinctive features of the Atlantic *Sebastes* sp. but confessed that it was impossible to distinguish the rosefish, *S. fasciatus*, from the sharp-beaked redfish, *S. mentella*, by external appearance. Litvinenko (1974) found the nature of coloration may serve as a useful character in identification of these two different types of sharp-beaked redfishes in the juvenile stage. Templeman (1980) examined the preextrusion larvae of redfishes and indicated that two types of sharp-beaked redfishes were found. However, none of these studies enabled identification of these two types of sharp-beaked redfishes with confidence.

Hallacher (1974, in an addendum) reported that Eschmeyer had examined a few specimens of the four species of North Atlantic redfishes and found that the gasbladder muscles passed between ventral ribs 2 and 3 in *S. marinus* and *S. mentella* and between ribs 3 and 4 in *S. fasciatus* and *S. viviparus*. Ni (1981) examined 200 sharp-beaked redfish specimens and concluded that the route of passage of the extrinsic gasbladder muscle through the ventral ribs was the one clear-cut discriminator between *S. fasciatus* and *S. mentella*. It passed between ventral ribs 2 and 3 in *S. mentella* and between ventral ribs 3 and 4 or 4 and 5 in *S. fasciatus*. This determination requires special skills and is not suitable to apply in a field survey to separate sharp-beaked redfishes. Therefore, univariate statistics and discriminant analysis were conducted on the same 200 sharp-beaked redfishes that had been separated on the basis of the extrinsic gasbladder muscle. Emphasis was on the application of discriminant analysis to determine good morphological discriminators which could be easily utilized in field studies. Good results from discriminant analysis, based on several combinations of meristic and morphological characters, again supported the existence of two different species of sharp-beaked redfishes. Guidelines for the separation of sharp-beaked redfishes are suggested.

Materials

During a research cruise in 1973, 200 specimens of sharp-beaked redfishes were collected from the eastern Grand Bank. Specimens were separated into two groups using the extrinsic gasbladder musculature. One hundred *S. fasciatus* ranged in standard length from 100 to 250 mm and 100 *S. mentella* ranged from 188 to 377 mm. For sample details refer to Ni (1981).

All fish were frozen and thawed prior to measurement. There were 11 meristic and 14 morphological characters recorded, which were mostly suggested by Barsukov (1972) and Barsukov and Zakharov (1972). Measurements taken and their corresponding abbreviations are listed in Appendix 1.

Statistical Analysis and Results

Univariate statistics of the morphological characteristics of *S. mentella* and *S. fasciatus* were calculated separately. The mean, sample size, and standard error of the mean are shown in Table 1. In univariate analysis, for all but small sample sizes, the probability values for the *t*-test of mean values are not greatly affected by moderate departures from normality. However, the distribution of the *F* statistics is sensitive to the assumption of normality and lacks robustness (Armitage 1971). Therefore, the alternative formulations of Levene's test (Levene 1960; Draper and Hunter 1969) were utilized for the test of equality of variances as it was found to be robust under conditions of nonnormality (Brown and Forsythe 1974). When the population variances of the two groups were not significantly different, the estimate of the variance was obtained by pooling (averaging) the two estimates of the variance for the *t*-test. If the population variances of the two groups were significantly different, the Welch–Aspin (separate variance) method was used whereby the variance of each group was estimated separately.

The results of the univariate tests (Table 1) showed that of the 11 meristic characters, 4 differed significantly in their variances and 10 differed significantly in their means ($P < 0.01$). The percentage frequency distribution of vertebrae, dorsal fin rays, pectoral fin rays, and anal fin rays is shown in Fig. 1. This illustrates the overlapping of meristics between *S. mentella* and *S. fasciatus* even when they are significantly different. Among the 14 morphological characters,7 showed significant differences in their variances and 11 showed significant differences in their means. In other words, significant differences were found between *S. mentella* and *S. fasciatus* in 11 variables from tests of variance and in 21 variables from tests of means out of 25 meristic and morphological characters.

After finding morphological differences between *S. mentella* and *S. fasciatus*, it was important to know whether each specimen could be identified as to species with-

TABLE 1. Sample sizes (n), mean values, and standard error (SE) of meristic and morphological characters for *Sebastes mentella* and *S. fasciatus*. Levene's test of variances and the appropriate t-test are at $P = 0.05$ (*) and $P = 0.01$ (**) significance levels. Abbreviations are defined in Appendix 1.

Meristic and morphological characters	*Sebastus mentella*			*Sebastes fasciatus*			Levene test	t-test
	n	$\bar{x}$	SE	n	$\bar{x}$	SE		
1. DORSARAY	100	14.67	0.055	100	13.62	0.055	ns	**
2. ANALRAY	100	8.62	0.057	100	7.02	0.014	**	**
3. PECTORAY	100	19.29	0.056	100	19.09	0.035	**	**
4. UPUBPTRA	99	1.96	0.040	100	1.95	0.026	ns	ns
5. TLBRPTRA	99	9.75	0.114	100	8.53	0.072	ns	**
6. LOUBPTRA	99	7.59	0.109	100	8.61	0.070	*	**
7. LATEPORE	99	34.25	0.111	100	33.13	0.083	ns	**
8. VERTEBRA	100	30.03	0.022	99	29.31	0.047	**	**
9. TLGILRAK	99	34.00	0.118	99	36.43	0.103	ns	**
10. LOGILRAK	99	24.21	0.092	99	25.72	0.084	ns	**
11. UPGILRAK	99	9.79	0.060	100	10.72	0.049	ns	**
12. AN1OPESP	96	−15.35	0.798	96	−13.39	0.851	ns	ns
13. AN3OPESP	95	60.11	1.235	99	32.79	1.037	ns	**
14. AN5OPESP	97	107.48	1.303	99	93.36	1.115	*	**
15. FUSIOCNU	98	1.49	0.098	100	3.04	0.116	**	**
16. PREOCUSP	100	1.15	0.054	100	1.00	0.0	**	**
17. PREOCUDG	98	1.41	0.078	100	2.15	0.809	ns	**
18. SUPOCUSP	100	1.45	0.090	99	1.00	0.0	**	**
19. SUPOCUDG	94	1.24	0.075	99	2.38	0.113	**	**
20. POSOCUSP	99	1.00	0.0	100	1.00	0.0	ns	ns
21. POSOCUDG	99	1.41	0.066	100	2.30	0.100	ns	**
22. TYMPANSP	98	1.02	0.014	100	1.00	0.0	**	ns
23. TYMPANDG	98	1.41	0.979	100	2.25	0.073	ns	**
24. CORONASP	100	3.99	0.010	100	3.66	0.091	**	**
25. PECTANUS	99	8.44	0.106	98	5.04	0.154	ns	**

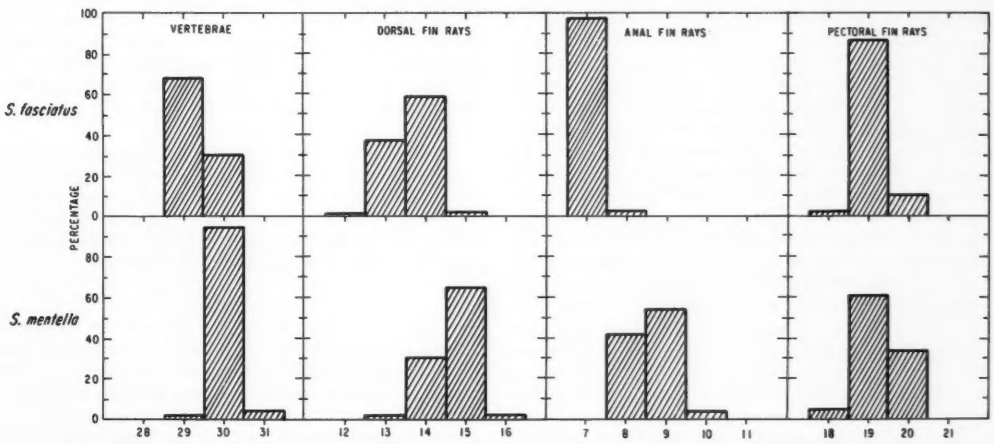

FIG. 1. Comparison of percentage frequency distribution of four meristic characters between *Sebastes mentella* and *S. fasciatus*.

out reference to extrinsic gasbladder masculature. This led to the use of discriminant analysis, the general object of which was to find the best character combinations for assigning specimens to the predefined groups. This was conducted on different combinations of meristic and morphological characters. The characters used in computing the linear classification functions were chosen in a stepwise manner. At each step the character that added most to the separation of the group was entered. Group classification was evaluated and presented in a classification table. In addition, a jackknife validation pro-

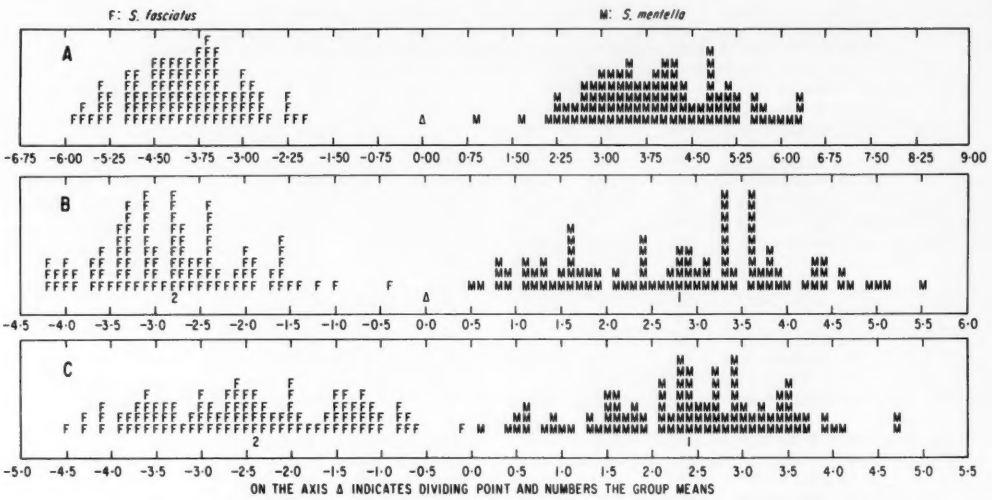

FIG. 2. Histograms of canonical variables in the discriminant analysis for different combinations of variables in *Sebastes mentella* and *S. fasciatus*. (A) 25 meristic and morphological variables; (B) 11 meristic variables subjected to square root transformation; and (C) 14 morphological variables.

cedure (Lachenbruch and Mickly 1968) was required to reduce the bias in the group classification and was computed from all the data except that of the specimen being classified. The BMDP7M program (Dixon and Brown 1977) was used to compute canonical variables and discriminant functions. The first canonical variable was the linear combination of variables entered that best discriminated among the groups. The second canonical variable was the next-best linear combination orthogonal to the first one. The canonical variables were adjusted so that the pooled within-group variances were one and the overall mean was zero. The canonical variables were evaluated as the group means. A plot of canonical variables gave an optimal picture of the separation of the group. The statistical theory of discriminant analysis assumes that the discriminating variables have multiple normal distribution and that they have equal variance–covariance matrices within each group. In practice, the technique is very robust and these assumptions need not be strictly adhered to (Klecka 1975).

The following analyses of combinations of meristic and morphological characters were conducted on 200 specimens.

A) The analysis was first computed using all 25 meristic and morphological variables, which resulted in 100% correct classification of the two groups. The number of anal fin rays was the most important character in discriminating groups, followed by relative position of tips of pectoral and pelvin fin to anus, total gill-raker number, angle of the third preopercular spine, and fusion of the occipital–nuchal ridge. The skewness and kurtosis of the 11 meristic variables were then examined by the g_1 and g_2 statistics. Square root transformation was done to meet the assumption of a multiple normal distribution. The analysis was repeated with the meristic characters transformed. One hundred percent separation was again obtained.

B) With the 11 transformed meristic characters, discriminant analysis and jackknife method showed that 100% separation could be obtained. The number of anal fin rays, total number of the first left gill rakers, vertebral number, number of branched left pectoral fin rays, and lateral line pores were the five important discriminators.

C) Using the 14 morphological characters, I correctly classified 100% of the specimens using discriminant analysis and 99.5% using the jackknife method. Good discriminators were the relative position of the pectoral fin and pelvic fin to anus, the angle of the third preopercular spine, fusion of the occipital–nuchal ridge, the degree of development of tympanic spines, and the degree of development of supraocular spines.

The histograms of the first canonical variables in the discriminant analysis for the above three combinations of variables are shown in Fig. 2. A summary table of the results is in Table 2. Only the first five variables entered into the equation are listed to show the sequential selection of variables that contributed most to the separation of the groups.

Discussion

Significant morphological differences between the two types of sharp-beaked redfishes were demonstrated in the univariate analysis. In addition, the discriminant analysis listed the good discriminators in order of importance. The meristic characters used served well as discriminators, particularly the number of anal fin rays, the number of rakers on the first left gill arch, the vertebral number, and the total number of branched pectoral fin rays. Morphological characters that were good in differentiating these two types were the relative position of pectoral and pelvic fins to anus, the angle of the third preopercular spine, the fusion of the occipital–nuchal ridge, etc. Morphological guidelines for the separation of the two species of sharp-beaked redfishes are given in Table 3.

TABLE 2. Results of discriminant analysis of three combinations of morphological characters. Characters entered show the variable that adds most to the separation of the groups in the order in which they entered the equation. Abbreviations are defined in Appendix 1.

	No. of variables used		
	(A) 25 (11 meristic + 14 morphological characters)	(B) 11 transformed meristics	(C) 14 morphological characters
Resubstitution classification	100%	100%	100%
Jackknifed classification	100%	100%	99.5%
First five characters entered	2. ANALRAY 25. PECTANUS 9. TLGILRAK 13. AN3OPESP 15. FUSIOCNU	2. ANALRAY 9. TLGILRAK 8. VERTEBRA 5. TLBRPTRA 7. LATEPORE	25. PECTANUS 13. AN3OPESP 15. FUSIOCNU 23. TYMPANDG 19. SUPOCUDG

TABLE 3. Morphological guideline for separation of *Sebastes fasciatus* and *S. mentella*. Although the percentages are listed in the parentheses, interpreters should be aware of possible geographic variation. Ambiguous specimens should be dissected so that the extrinsic gasbladder musculature can be examined.

Morphological character	*S. fasciatus*	*S. mentella*
Anal fin rays	Mostly 7 (98%), occasionally 8 (2%)	8 or more (100%)
Tip of pectoral fin	Usually not reaching the anterior edge of anus (67%)	Mostly extends beyond the anus (98%)
First left gill rakers	Usually 36 or more (81%)	Usually 35 or less (90%)
Downward angle of the 3rd preopercular spine	Usually less than 50° (96%)	Usually more than 50° (82%)
Occipital–nuchal ridge	Usually fused on both sides (75%)	Usually separated on one or both sides (83%)
Route of extrinsic gasbladder muscle through ventral ribs (Ni 1981)	Between 3 and 4 or 4 and 5 (100%)	Between 2 and 3 (100%)

The separation was clear and was on the basis of characters which can be easily seen in the field situation. Only with occasional ambiguous specimens would the examination of the extrinsic gasbladder musculature be necessary.

Morphological characters play a very important role in species identification and stock discrimination. However, a standard coding system for the degree of development of head spines, relative position of tips of pectoral and pelvic fin to anus, coloration, etc., is not available. This makes it very difficult to compare results with those of other researchers. Morphometrics were avoided in this study as the specimens of *S. fasciatus* were relatively smaller than those of *S. mentella*.

It is difficult to obtain enough specimens from the same locality of the three different species of redfishes in the Northwest Atlantic, *S. marinus*, *S. mentella*, and *S. fasciatus*, for comparisons. As they are probably different species, they should occupy somewhat different ecological niches. A multivariate classification function for *S. mentella* and *S. fasciatus* was developed based on the available data. Morphological information on *S. marinus* would be necessary to demonstrate that this redfish differs from the others.

Morphological studies based on 11 meristic and 14 morphological characters again substantiate the existence of two different species of sharp-beaked redfishes, *S. mentella* and *S. fasciatus*. We must then consider the possible implications for redfish management resulting from the recognition that those two different species exist. The morphological features useful in differentiating redfishes should be analyzed in

other regions with a view to solving the redfish species composition problem.

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Appendix 1. Methods of Measurements

A) MERISTICS

1.	DORSARAY	Number of dorsal fin rays
2.	ANALRAY	Number of anal fin rays
3.	PECTORAY	Number of left pectoral fin rays
4.	UPUBPTRA	Number of upper unbranched left pectoral fin rays
5.	TLBRPTRA	Number of total branched left pectoral fin rays
6.	LOUBPTRA	Number of lower unbranched left pectoral fin rays
7.	LATERPOR	Number of lateral line pores on left side of fish
8.	VERTEBRA	Number of vertebrae (urostyle excluded)
9.	TLGILRAK	Total number of gill rakers on first left gill arch
10.	LOGILRAK	Number of gill rakers on the lower proportion of first left gill arch including the raker straddling the angle of the arch
11.	UPGLIRAK	Number of gill rakers on upper portion of first left gill arch excluding the raker straddling the angle of the arch

B) MORPHOLOGICAL CHARACTERS

12.	AN1OPESP	Angle of the first preopercular spine from the horizontal axis
13.	AN3OPESP	Angle of the third preopecular spine from the horizontal axis
14.	AN5OPESP	Angle of the fifth preopercular spine from the horizontal axis
15.	FUSIOCNU	Degree of fusion of the occipital–nuchal ridge according to the following code 1. Fused both sides 2. Fused left side, separated right 3. Fused right side, separated left 4. Separated both sides
16.	PREOCUSP	Development of preocular spines as follows 1. Present both sides 2. Present left, absent right 3. Present right, absent left 4. Absent both sides
17.	PREOCUDG	Degree of development of preocular spines as follows 1. Weak, both sides (or one if absent on other) 2. Moderate both sides (or one if absent on other) 3. Well-developed (strong) both sides 4. Weak left, moderate right 5. Weak right, moderate left 6. Strong right, moderate left 7. Strong left, moderate right
18.	SUPOCUSP	Development of supraocular spines coded as 16
19.	SUPOCUDG	Degree of development of supraocular spines coded as 17
20.	POSOCUSP	Development of postocular spines coded as 16

21. POSOCUDG Degree of development of postocular spines coded as 17
22. TYMPANSP Development of tympanic spines coded as 16
23. TYMPANDG Degree of development of tympanic spines coded as 17
24. CORONASP Development of coronal spines coded as 16
25. PECTANUS Relative positions of tips of left pectoral and left pelvic fin to anus; use the following code
    1. Tip of pectoral extends about one-third or less of distance between tips of pelvic and anterior edge of the anus.
    2. Tip of pectoral extends to about midway between tip of pelvic and the anterior edge of the anus.
    3. Tip of pectoral extends to two thirds of the distance between tip of pelvic and anterior edge of the anus.
    4. Tip of pectoral extends to more than two-thirds the distance between tip of pelvic and anterior edge of the anus.
    5. Tip of pectoral extends to the anterior edge of the anus
    6. Tip of pectoral extends beyond the anterior edge of the anus
    7. Tip of pectoral extends to the midpoint of the anus
    8. Tip of pectoral extends to the midpoint of the anus
    9. Tip of pectoral extends beyond the posterior edge of the anus

Acclimation to Copper by Rainbow Trout (*Salmo gairdneri*) — A Modifying Factor in Toxicity

D. GEORGE DIXON[1] AND JOHN B. SPRAGUE

Department of Zoology, College of Biological Science, University of Guelph, Guelph, Ont. N1G 2W1

DIXON, D. G., AND J. B. SPRAGUE. 1981. Acclimation to copper by rainbow trout (*Salmo gairdneri*) — a modifying factor in toxicity. Can. J. Fish. Aquat. Sci. 38: 880–888.

Lethal tolerance to copper by rainbow trout (*Salmo gairdneri*) increased by 60, 106, and 90% following 3-wk exposures to 0.29, 0.40, and 0.59 of the mean control incipient lethal level (ILL), 330 μg Cu/L (SD = 30; n = 25). A lower level of exposure, 0.18 ILL, represented a threshold for the onset of acclimation as tolerance was unaffected. Exposure at 0.09 ILL sensitized the trout, reducing their tolerance by 20%. The three-dimensional response surface for tolerance thus resembled a ski jump, with increases in tolerance for both duration and concentration of exposure above the threshold and sensitization below it. Because of the graded response, lethal tests on acclimated fish could be useful for rapid initial estimates of sublethal thresholds of response. Impact on growth was almost opposite to change in tolerance. Growth was reduced at 0.29, 0.40, and 0.59 ILL, enhanced at 0.18 ILL, and unaffected at 0.09 ILL. Acclimation to copper was not retained. That induced by 0.40 ILL was gradually lost during 3 wk in control water. A 4-wk exposure of fish to 0.59 ILL of copper resulted in sensitization to lethal levels of zinc. Tolerance to zinc decreased by 56%, the ILL of zinc dropping from 6.2 to 2.7 mg/L.

Key words: tolerance, resistance, acclimation, copper, zinc, rainbow trout, *Salmo gairdneri*; toxicity

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La tolérance au cuivre de la truite arc-en-ciel (*Salmo gairdneri*) jusqu'au seuil de létalité, a augmenté de 60, de 106 et de 90 % après exposition de 3 semaines à 0,29, à 0,40 et à 0,59 fois la concentration moyenne où on observe un début de mortalité chez les témoins (CDM), 330 μg de Cu/L (SD = 30; n = 25). À une concentration inférieure, 0,18 CDM, se trouvait le seuil du début de l'acclimatation, étant donné que la tolérance n'était pas modifiée. L'exposition à 0,09 CDM a sensibilité la truite, réduisant sa tolérance de 20 %. La surface tridimensionnelle de la tolérance ressemblait ainsi à un saut, la tolérance augmentant selon la durée et la concentration d'exposition au-dessus du seuil et la sensibilisation au-dessous. À cause de ces réactions graduelles, des essais de létalité effectués sur des poissons acclimatés pourraient aider à obtenir une première estimation rapide des seuils sublétaux. L'influence sur la croissance a été presque à l'opposé des modifications de la tolérance. La croissance a été réduite à 0,29, à 0,40 et à 0,59 CDM, a augmenté à 0,18 CDM et est restée la même à 0,09 CDM. L'acclimatation au cuivre n'a pas persisté. Celle qui a été obtenue par exposition à 0,40 CDM s'est graduellement résorbée au cours du séjour de 3 semaines dans l'eau témoin. Le poisson exposé 4 semaines à 0,59 CDM de cuivre a été sensibilisé à des concentrations létales de zinc. La tolérance au zinc a diminué de 56 %, la CDM du zinc chutant de 6,2 à 2,7 mg/L.

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ACCLIMATION by aquatic organisms to different levels of "natural" variables in their environment has long been recognized. A particularly complete picture has been developed on the relationship between lethal temperature and acclimation temperature for fish (summarized by Fry 1971). Acclimation-induced changes in tolerance have also been documented for dissolved oxygen (Shepard 1955; Moss and Scott 1961), carbon dioxide (Saunders 1962; Lloyd and White 1967), and salinity (McLeese 1956).

The question of acclimation by fish to toxicants has never been fully investigated. This is surprising as resulting changes in tolerance could be of considerable importance in research carried out for the purpose of setting water quality criteria.

[1]Present address: Department of Fisheries and Oceans, Great Lakes Biolimnology Laboratory, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6.

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Laboratory experiments to test the effects of sublethal levels of toxicants invariably use fish which were previously held in clean water. Results obtained under these conditions could be in error when applied to wild fish which had adapted to low-level pollution. In particular, it may be unclear whether physiological changes during a sublethal exposure represent deleterious effects of the toxicant or merely the normal adaptive mechanisms of the organism.

Instances of increased resistance to toxicants following acclimation have been noted in a number of studies (Lloyd 1960; Lloyd and Orr 1969; Hokanson and Smith 1971; Sinley et al. 1974; Spehar 1976). The picture is fragmentary as the research was generally carried out for other purposes. Furthermore, most of these studies reported results in terms of change in median lethal time (resistance, Fry 1947) instead of change in the more physiologically meaningful threshold lethal concentration or incipient lethal level (ILL) (tolerance, Fry 1947). In a previous paper (Dixon and Sprague 1981) we showed that there were indeed changes in ILL of arsenic and cyanide when rainbow trout (*Salmo gairdneri*) were acclimated to a sublethal level of either toxicant. That initial work did not, however, attempt to show whether the degree of change in ILL was related to the acclimation concentration.

The present study was designed to investigate changes in toxicant tolerance shown by rainbow trout following acclimation to that toxicant over a range of sublethal concentrations for varying periods of time. Copper was chosen for the experiments as a representative and widespread pollutant which gave promising results during preliminary tests. Retention of acquired tolerance was also studied following transfer of the fish back to control water. Specificity of the response was checked by determining tolerance of copper-acclimated fish to zinc.

Materials and Methods

Rainbow trout parr from Goossens Trout Farm Ltd., Otterville, Ont., with initial mean wet weights ranging from 1.7 to 3.3 g, were used in these experiments. Fish were held in the laboratory water for a minimum of 4 wk prior to experiments, with mortality of 0.5% or less. Volume of holding tanks and flow rate of new water exceeded usual guidelines (Sprague 1969). Conditions of temperature, dissolved oxygen, photoperiod, and feeding were the same as those maintained during experiments.

Experiments were carried out using two 170-L acclimation tanks and twelve 15-L bioassay tanks, all constructed from unplasticized polyethylene. A continuous flow of water was supplied to each tank from a constant-head source using flowmeters (Manostat Corp., New York). Each acclimation or bioassay tank received, respectively, 2.0 or 0.2 L of water per minute for a 95% molecular replacement time of 4.5 h (Sprague 1973). Mariotte bottles (Leduc 1966) were used to dispense toxicant solution into the water streams. Toxicant solutions were prepared with deionized water and reagent grade $CuSO_4 \cdot 5H_2O$ or $ZnSO_4 \cdot 7H_2O$, and acidified with 10 mL of 5 mol/L H_2SO_4 per 20 litre. Photoperiod was 14 h light and 10 h dark, with the first 0.5 h of each period occupied by brightening or dimming; intensity from mixed incandescent and "daylight" flourescent lights was 20–30 lx at the water surface.

TABLE. 1. Chemical characteristics of laboratory water with standard deviations and number of measurements during the study. All units are milligrams per litre except for specific conductance, which is microsiemens per centimetre, and pH.

Parameter	Mean	Standard deviation	No. of observations
Acidity (as $CaCO_3$)	8.3	0.6	81
Alkalinity (as $CaCO_3$)	223	4.8	261
Ammonia	0.03	0.01	3
Cadmium	<0.0005	—	9
Calcium	77	2.6	9
Chloride	30.2	0.08	9
Chromium	<0.005	—	9
Copper	0.003	0.0019	21
Fluoride	0.96	0.01	9
Iron	0.12	0.0028	9
Lead	<0.001	—	9
Magnesium	43	1.9	9
Nickel	<0.002	—	9
Nitrate	0.58	0.49	9
pH	7.75	0.04	311
Phosphate	<0.1	—	9
Specific conductance	610	80	14
Sulfate	129	1.9	9
Total hardness (as $CaCO_3$)	374	9.8	318
Zinc	0.019	0.0027	9

The laboratory water, from wells, was hard but otherwise of normal composition (Table 1). Water was untreated, except for aeration and filtration through polyester fiber. Throughout experiments, in-tank temperatures averaged 15.0°C (SD = 0.08), dissolved oxygen 8.6 mg/L (SD = 0.3), and carbon dioxide did not exceed 4.8 mg/L. The only major fluctuation in water quality occurred during acclimation and recovery at 131 μg Cu/L. On day 17, total hardness dropped from 376 to 310 mg/L. The level then increased continuously, reaching 360 mg/L at termination.

Copper or zinc concentrations were measured in daily samples from each acclimation and bioassay tank. After filtration through a 0.45-μm membrane filter (Hem 1972), samples were acidified (HNO_3 to 1%) and stored in linear polyethylene bottles. Metals were analyzed by atomic absorption spectrophotometry (Pye Unicam model SP 1950). Copper above 200 μg/L and zinc were measured directly by flame volatilization of samples. This technique gave detection limits and sensitivities of 100 and 2 μg/L for copper and 100 and 50 μg/L for zinc. Lower copper concentrations were preconcentrated by organic chelation and solvent extraction (Kinrade and Van Loon 1974), which increased the detection limit to 10 μg/L. All metal concentrations reported are average measured values for that tank, as filtered Cu^{2+} or Zn^{2+}.

Separate groups of fish were acclimated to five sublethal levels of copper for 3 wk and changes in tolerance of lethal levels of the metal were measured by bioassay. The experiments were conducted during the period from October 1977 to April 1979. To begin an experiment, a minimum of 480 fish were transferred from holding to an acclimation tank receiving clean water. Acclimation to copper was started 1 wk after transfer. In the five experiments fish were acclimated to average measured concentrations of 30, 58, 94, 131, and 194 μg Cu/L (SD = 5, 4, 7, 18, and 11 respectively). Those

levels corresponded to 0.09, 0.18, 0.29, 0.40, and 0.59 of the mean control ILL of copper, 330 μg/L. Fish were fed once daily by hand during acclimation, at a constant rate of 2.5% of the biomass at time of transfer. Ration level was corrected for the periodic removal of fish but not for growth. The Silver Cup juvenile salmonid diet contained 7.4–9.7 mg Cu/kg of feed. Fish were not fed during bioassays.

On days 0, 7, 14, and 21 of acclimation, 120 fish were removed from the acclimation tank and used in a copper bioassay. The fish were randomly distributed, 20 per tank, to five concentrations of copper plus control. Mean measured concentrations of copper, used in calculating LC50s, were 83–95% of nominal and had coefficients of variation ≤8.2%. Mortalities were observed on a logarithmic time sequence up to a maximum of 144 h. The dry weights of all bioassay fish were taken after freeze-drying. Size attained by fish used in successive bioassays provided information on the effects of copper on growth during acclimation.

Retention of increased tolerance, after return to clean water, was studied by continuing the experiment for acclimation to 131 μg/L. Copper flow to the acclimation tank was terminated at 21 d and bioassays were started after a further 7, 14, and 21 d. Only 10 fish per concentration were used in these later bioassays.

The impact of copper acclimation on zinc tolerance was investigated in conjunction with acclimation to 194 μg Cu/L. The 21-d acclimation to copper was extended for an additional 7 d, after which a bioassay with zinc was conducted. Mean measured concentrations of zinc were 100–109% of nominal with coefficients of variation ≤8.8%.

In all of the above acclimation experiments, an equally large group of control fish was simultaneously subjected to exactly the same procedures, except that the dosing apparatus delivered stock solutions containing no copper to the tanks during acclimation. This allowed each bioassay with acclimated fish to be compared with a simultaneous control on unacclimated fish.

Data Analyses

Median lethal concentrations (LC50s) were calculated by computerized probit analyses (Finney 1971). Correction for control mortality was unnecessary as none occurred during the 48 bioassays. Significant differences between LC50s were assessed by standard error of the difference (Sprague and Fogels 1977) or relative potency (Finney 1971). A response surface for copper tolerance was established by computerized multiple regression (Steel and Torrie 1960). Differences in mean postbioassay dry weights of fish were distinguished by Tukey's comparison of means (Steel and Torrie 1980). Compliance of all data with assumptions of normality and homogeneity of variability was established prior to analysis. The statistical significance of all results was assessed at the 0.05 probability level.

Specific growth rates were calculated (Brown 1957), from mean dry weights of the fish in successive bioassays, for each 7-d interval of acclimation. As this involved discrete samples of fish, as opposed to successive measurements on the same fish, statistical analysis was not possible. The specific growth rates are presented solely to give indications as to which periods of copper acclimation had the greatest impact on growth, with no statistical inference implied.

Results

Thresholds of lethality were attained in 60–96 h in all bioassays; this paper accordingly deals with incipient lethal levels (ILL). Thresholds consistently occurred earlier in groups of fish that showed acclimation to copper than in controls (Dixon 1980). Mortality of 1.25% was sustained during acclimation to 194 μg/L; none occurred in any other control or acclimated group. The mean ILL for the 20 control bioassays was the same as the mean for the 5 acclimation bioassays at time zero: 330 μg Cu/L. Thus 25 bioassays indicate that the ILL of copper for rainbow trout in our laboratory water is 330 μg/L, with a standard deviation of 30.

Copper Acclimation

Acclimation to 94, 131, and 194 μg Cu/L resulted in significant increases in lethal tolerance to copper by rainbow trout. At these three highest levels of acclimation, the ILLs for acclimated fish were significantly greater than their simultaneous controls for all acclimation times of 7, 14, and 21 d (Table 2). By 21 d, ILLs had increased relative to their controls by 60, 105, and 90% to levels of 513, 564, and 708 μg/L. Most of the increase in tolerance occurred during the first 7 d of acclimation at these three highest levels. Although there appeared to be trends for increasing tolerance

TABLE. 2. Incipient lethal levels of copper obtained with control and copper-acclimated rainbow trout. All bioassays continued for 144 h. Units are micrograms per litre for levels of acclimation, ILL, and fiducial limits, and days for duration of acclimation. The slope is that of the fitted probit line.

Acclimation		Acclimated fish		Control fish	
Level	Duration	ILL (fiducial limits)	Slope	ILL (fiducial limits)	Slope
30	0	302 (240–357)	6.7	294 (233–349)	6.5
	7	280 (235–324)	6.9	328 (281–378)	7.1
	14	249 (206–290)	7.2	320 (273–368)	6.8
	21	266 (224–308)	6.6	329 (283–379)	7.3
58	0	299 (260–340)	6.6	305 (265–346)	6.0
	7	310 (263–352)	8.5	320 (273–363)	6.2
	14	344 (304–389)	7.1	335 (296–378)	7.4
	21	349 (307–395)	6.1	333 (293–378)	8.7
94	0	316 (232–431)	3.9	328 (256–419)	5.2
	7	450 (388–520)	6.1	304 (212–438)	3.6
	14	472 (426–525)	9.4	324 (248–424)	4.3
	21	515 (457–580)	7.9	311 (218–442)	3.7
131	0	374 (309–453)	5.6	360 (290–446)	5.3
	7	639 (585–699)	10.9	381 (323–450)	6.2
	14	616 (558–681)	8.5	298 (214–417)	4.0
	21	564 (508–625)	7.9	274 (237–317)	10.6
194	0	367 (305–443)	6.2	371 (308–448)	6.0
	7	681 (621–747)	10.7	369 (308–442)	6.3
	14	717 (656–783)	12.9	365 (303–441)	5.7
	21	708 (648–771)	10.8	371 (315–437)	6.1

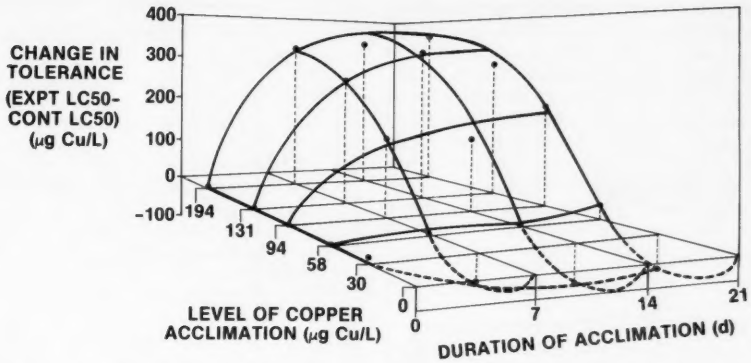

FIG. 1. A response surface to illustrate the change in incipient lethal level of copper for rainbow trout acclimated to any combination of (a) copper from 0 to 194 μg/L with (b) time from 0 to 21 d. Observed values are points, predicted values intercepts.

between 7 and 21 d of acclimation (Table 2, Fig. 1), the changes were not large enough to show any significant differences among ILLs for 7, 14, and 21 d of acclimation within experiments at these concentrations.

Acclimation to 58 μg/L resulted in no significant changes in ILL relative to controls, i.e. no change in tolerance was

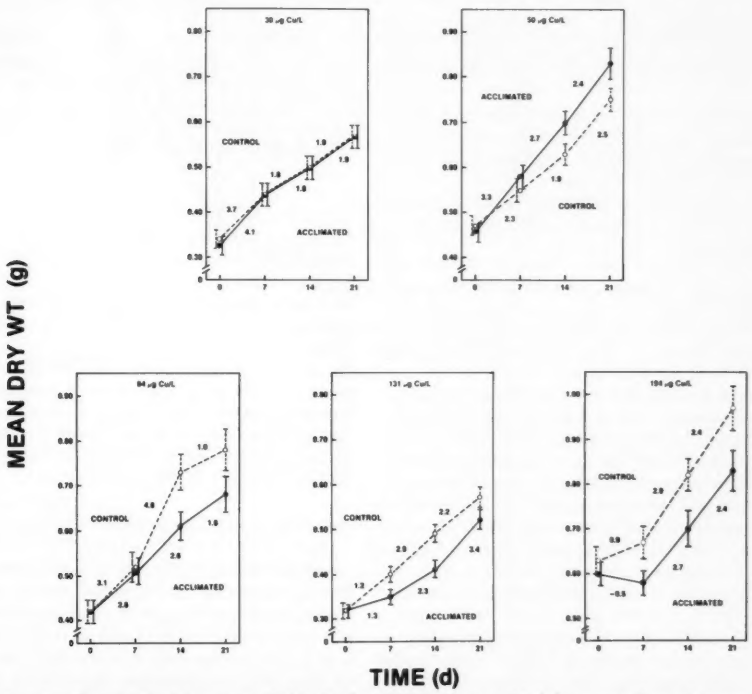

FIG. 2. Growth of rainbow trout during 21 d of acclimation to sublethal concentrations of copper compared with growth of fish in control water. Acclimation concentrations are given at the top of each panel. The 95% confidence intervals are shown for the mean postbioassay dry weights. Numerical values alongside the lines are specific growth rates (percent dry weight per day) for that 7-d period.

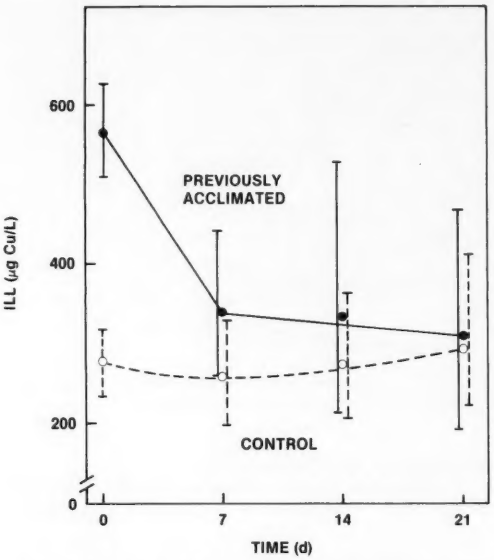

FIG. 3. Loss of acclimation to copper in fish returned to clean water after a 21-d exposure to 131 µg Cu/L. Time zero represents transfer to clean water. Each point is an ILL with its 95% fiducial limits.

evident (Table 2, Fig. 1). At 30 µg/L, however, acclimation resulted in decreased tolerance. After 14 and 21 d ILLs were 249 and 266 µg/L for acclimated fish, significantly lower than the control values of 320 and 329 µg/L.

The response surface for the difference between each experimental ILL and its simultaneous control over the design region was calculated as:

$$D = \text{antilog}\,(2.30 + 1.73A + 0.17T - 0.97A^2 - 1.02T^2 + 2.00AT - 3.01A^3 + 9.33T^3 - 3.38A^2T - 9.92T^2A) - 100$$

where D = experimental Cu ILL − control Cu ILL in micrograms per litre; $A = (\log_{10} A') - 1.924$, A' being the acclimation concentration in micrograms per litre; and $T = \log_{10}(T' + 10) - 1.276$, T' being the duration of acclimation in days. The transformations of acclimation concentration (A) and duration (T) were necessary to center the data, thus preventing autocorrelation within the analysis. The relationship was statistically significant, with the equation accounting for 99.3% of the variability in response. Residuals gave no indication of an unequal predictive ability over the response surface.

The response surface (Fig. 1) resembles a ski jump in configuration, leveling to a plateau at the two highest levels of acclimation. The figure represents in a unified way the changes described above: reduced ILLs after acclimation to 30 µg/L; no effect at 58 µg/L; increased ILLs at 94 µg/L; and further increase to a plateau of tolerance following acclimation to 131 and 194 µg/L.

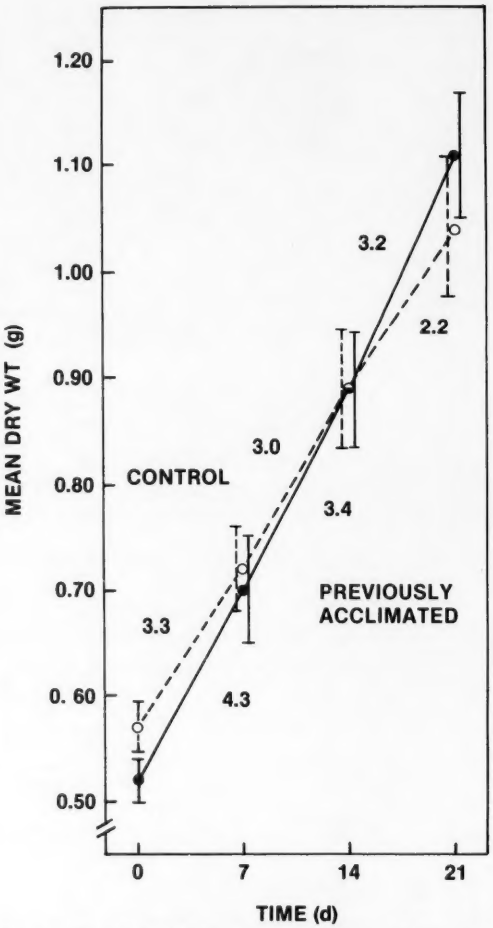

FIG. 4. Growth of rainbow trout in control water after removal from copper solution. The values are mean dry weights attained by fish used in successive bioassays. The 95% confidence intervals are given. Values alongside the lines are specific growth rates (percent dry weight per day) for that 7-d period.

GROWTH DURING ACCLIMATION

Fish acclimated to copper at 30 µg/L showed no significant alteration in attained dry weight relative to controls (Fig. 2). Fish acclimated to 58 µg/L attained significantly greater size than their controls after 14 and 21 d. Specific growth rates (Fig. 2) suggest that the effect of copper was greatest during the first 14 d of acclimation. Acclimation to 94, 131, and 194 µg/L resulted in smaller attained sizes. In fish acclimated to 94 µg/L, mean dry weights were significantly lower than those of controls at 14 and 21 d. Acclimation to 131 µg/L resulted in lower weights after 7, 12, and 21 d. The same was

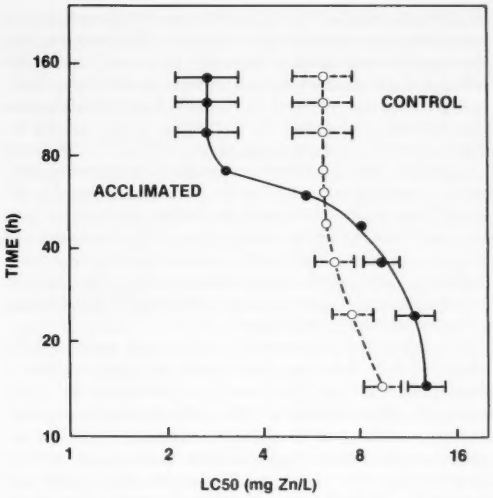

FIG. 5. Toxicity curves for zinc bioassays obtained with rainbow trout acclimated to 194 μg Cu/L or clean water for 28 d. The 95% fiducial limits are given for selected LC50s; those omitted were not significantly different.

true for fish acclimated to 194 μg/L but the response was more pronounced and mean weight actually decreased during the first 7 d of exposure. Based on specific growth rates (Fig. 2), the depression in weight gain appeared to be greatest during the initial period of acclimation to copper.

Loss of Increased Tolerance

The increased tolerance to copper developed on acclimation was not a sustained response. When fish were returned to control water for 21 d, after a 3-wk exposure to 131 μg Cu/L, they showed a continuing decrease in ILL relative to controls (Fig. 3). The ILLs for time zero in Fig. 3 are actually the values obtained for 21 d of acclimation and, as described above, the acclimated fish were significantly more tolerant than their controls. After 7, 14, and 21 d of recovery, however, the ILLs for the three bioassays with acclimated fish and the three with control fish were all similar, ranging from 356 to 340 μg Cu/L, with no significant differences apparent. It appears that most of the loss in acclimation occurred during the first 7 d in clean water.

There were no significant differences between the mean attained weights of control and exposed fish from 7 days onward in the experiment (Fig. 4). Although acclimated fish were smaller than controls at the end of copper exposure (time zero on Fig. 4), their specific growth rate was consistently higher during the period in clean water. This negated the initial difference over the course of the experiment.

Zinc Tolerance of Copper-Acclimated Fish

Fish acclimated to copper did not develop increased tolerance to zinc. The ILL of zinc for control fish was 6.2 mg/L, significantly higher than the value of 2.7 mg/L obtained with fish acclimated to 194 μg Cu/L for 28 d. There were substantial differences in toxicity curves for the two groups of fish (Fig. 5). Fish acclimated to copper showed greater resistance to zinc during the first 48 h of exposure. These short-term LC50s were significantly different from control values. During the 48 to 96-h period, however, acclimated fish suffered increased mortality, reaching a threshold in 96 h, later than the 60 h required by control fish. The mean dry weights of the control and acclimated bioassay fish were not significantly different at 1.01 and 0.97 g, respectively.

Discussion

Acclimation of rainbow trout to sublethal levels of copper had a marked effect on their lethal tolerance. The effect was dependent on the concentration used for acclimation, with a discrete threshold of no effect at 0.18 of the control ILL. Fish acclimated to concentrations above the threshold developed increased tolerance whereas those acclimated below the threshold showed reduced tolerance. Obviously there was a physiological mechanism functioning within the fish to mitigate the toxic effects of copper, and a minimum level of copper was required to activate that mechanism. At the threshold, the absence of altered tolerance suggests that the degree of induction of the adaptive mechanism was just sufficient to balance the detrimental effect of the previous copper exposure. Below the threshold, the presence of sensitization (an acclimation-induced reduction in tolerance) implies that induction did not occur and that some deleterious effect of acclimation was carried over that contributed to the impact of the subsequent lethal concentration. Fish which developed increased tolerance on acclimation did so rapidly, predominantly during the first 7 d of exposure.

The response surface for tolerance indicates a plateau at the two highest concentrations of acclimation after 14 and 21 d. This plateau suggests an upper ceiling of tolerance, a maximum capacity of physiological performance by the adaptive mechansim. The mean LC50 on the plateau, 658 μg/L, which represents an increase of 100% over the mean unacclimated LC50 of 330 μg/L, is thought to represent a reasonable estimate of the ultimate incipient lethal level (Fry 1947) for copper in our water. A twofold increase in tolerance after acclimation is similar in magnitude to responses shown for temperature acclimation (Brett 1952; Fry 1971) and low-level oxygen acclimation (Shepard 1955; Moss and Scott 1961).

The mean control ILL, 330 μg/L, falls at the lower end of the 300–1000 μg/L range reported for salmonids in extremely hard water (Spear and Pierce 1979). This could be due in large part to the use of filtered (= dissolved) values in calculating ILLs whereas some reports in the literature may use unfiltered (= total) values (Hem 1972).

There are no reports in the literature of enhanced tolerance or resistance to copper by fish after acclimation in the laboratory. Peterson (1974) acclimated brook trout (*Salvelinus fontinalis*) to copper at 0.15, 0.25, and 0.50 ILL for a 3-mo period. In lethal tests at 1.7 ILL, fish acclimated to 0.25 ILL showed a 30% increase in median lethal time (ET50) relative to controls, but the experimental design was not sufficiently powerful to indicate a significant difference. No effect was

apparent for 0.15 or 0.50 ILL. Some work with invertebrates has indicated increased resistance, but not tolerance, following toxicant acclimation. After acclimation to copper levels from 25 to 100 μg/L, the ET50 of copper for the brine shrimp *Artemia salina* increased significantly in lethal concentrations of 1 and 5 mg/L (Saliba and Ahsanullah 1973). However, no significant change in resistance was apparent at lethal levels from 10 to 100 mg/L. A similar trend was apparent for the polychaete *Ophryotrocha labronica* (Saliba and Ahsanullah 1973). In a further study Saliba and Krzyz (1976) demonstrated that resistance developed by *A. salina* was proportional both to length and concentration of copper acclimation.

The physiological adaptation reported here could be confounded in field studies by evolutionary adaptation over several generations. Skidmore (1964) has suggested that reports of enhanced tolerance of organisms from polluted environments could be due to selective mortality of the naturally less tolerant members of the populations studied. Polychaetes (*Nereis diversicolor*) from sites of high copper contamination were four times more tolerant to copper than worms from sites of low contamination (Bryan 1974). This tolerance was probably based on genetic selection over several generations as tolerance was neither readily lost by tolerant worms nor gained by nontolerant worms (Bryan and Hummerstone 1971, 1973). Midge larvae *Chironomus tentans* from sediments contaminated with copper exhibited significantly elevated growth and survival rates relative to larvae from "clean" sediments when organisms from both populations were reared in a contaminated medium (Wentsel et al. 1978).

None of the above reports suggest the presence of a threshold of response comparable to that reported here. The only indication of a threshold comes from work on zinc resistance in algae. Populations of *Stigeoclonium tenue* from sites having a zinc level greater than 200 μg/L have been shown to exhibit increased resistance to zinc relative to populations from sites with zinc levels below 200 μg/L (Harding and Whitton 1976; Whitton 1970). Two hundred micrograms per litre may represent a threshold of induction analogous to the threshold reported here.

Three aspects of the growth of fish acclimated to copper warrant discussion: (1) growth effects were almost the reverse of tolerance effects; (2) copper impact, in terms of specific growth rate, was greatest during the initial period of each experiment; and (3) growth was enhanced in fish acclimated to 0.18 ILL. The only level of acclimation that did not affect growth was 0.09 ILL, the concentration that sensitized the fish rendering them less tolerant. This level is generally recognized as a "safe" level of copper for salmonids (Spear and Pierce 1979; U.S. Environmental Protection Agency 1976). Increased tolerance occurred only above the "safe" level and in each case was accompanied by significantly reduced growth. Except at acclimation to 0.59 ILL, where the fish ceased feeding for the first 4 d of exposure, loss of appetite did not appear to be involved. This reinforces the concept of an inducible tolerance mechanism. Warren (1971) defines the scope for growth of an organism as the difference between the energy content of the diet and the energetic cost of all activities other than growth. A stressing factor such as a toxicant can incur energetic costs, with a concurrent reduction in energy available for growth. The reduced growth noted here was possibly the result of an elevated demand associated with establishing the mechanisms responsible for tolerance. The fact that the depression of growth was greatest during the initial period of each experiment implies that although considerable energy was required to establish the mechanism, less was required to maintain it, accounting for the growth rebound as the experiments progressed. Lett et al. (1976) noted that growth rates of rainbow trout exposed to copper concentrations ranging from 0.17 to 0.51 ILL were initially depressed but within 40 d approached values observed for control fish. Similar results were reported by Waiwood and Beamish for both growth (1978a) and critical swimming performance (1978b). This further demonstrates the ability of trout to adapt to copper and is consistent with the development of tolerance demonstrated here.

The stimulation of growth in fish during acclimation to copper at 0.18 ILL was most probably a manifestation of "sufficient challenge," a concept reviewed for the mammalian literature by Smyth (1967). The stimulation occurred at the same level as the initial development of tolerance. Whereas fish acclimated to this level were no more tolerant than controls, they were more tolerant than those acclimated at 0.09 ILL. Although there are several reports of improved growth of fish under slight stress from copper and other toxicants (Pickering 1968; Pickering and Gast 1972; McLeay and Brown 1974; Holdway and Sprague 1979), the physiological basis of the enhanced performance is unknown.

Increased tolerance resulting from copper acclimation was quickly lost on return of the fish to control water, most of it by 7 d. Loss of tolerance is consistent with a physiological mechanism induced by a specific stress, in this case copper. Removal of the stress which triggered the original adaptation makes that adaptation redundant; retention could be an energetic detriment. Retention of elevated tolerance would be of primary practical importance when considering the impact of fluctuating levels of a pollutant. On the basis of this work, and for copper at least, physiologically based increases in tolerance, resulting from exposure to one pulse of pollution by a toxicant, would afford little protection from a second pulse, unless an appreciable base-line level was maintained throughout.

Fish acclimated to copper were significantly less tolerant of lethal levels of zinc than were controls. It would appear that the adaptive mechanism induced by copper did not protect fish from zinc but resulted in sensitization. Although the copper-acclimated fish were initially more resistant, this gave way to sensitization after ~60 h. The lack of cross-acclimation (acclimation to one toxicant resulting in increased tolerance to a second) appears to be consistent with the literature. Although no information on fish is available, cross-acclimation to metals appears to be rare in both plants (McNeilly 1968) and polychaetes (Bryan 1974). As fish acclimated to zinc develop increased resistance to zinc (Lloyd 1960; Edwards and Brown 1966), the absence of a cross-acclimation suggests that the mechanisms responsible are metal specific.

Altered tolerance on acclimation to toxicants has definite environmental implications. Failure of attempts to stock salmonids into waters with elevated metal levels, when seemingly healthy salmonid populations occurred in waters under similar

conditions (Goodman 1951; Davies and Woodling 1978), could be partially explained by the development of enhanced tolerance. Schofield (1965) demonstrated that acclimation to zinc in a hatchery made it possible to stock brook trout into zinc-polluted waters.

The methods outlined here for demonstrating acclimation to a toxicant could be useful for estimating no-effect levels of aquatic pollutants. Altered tolerance to copper by rainbow trout was found to be dependent on acclimation concentration and time, the response ranging from sensitization at 0.09 ILL to a twofold increase in tolerance at 0.59 ILL. This type of concentration-dependent response is the basis of all experimental design used in determining the impact of a toxicant, whether the measured response is survival, growth, reproductive success, avoidance of toxicants, or altered tolerance. The presence of a discrete threshold of increased tolerance would represent the concentration of onset of sublethal toxicity, with sensitization below that threshold being equivalent to the sublethal no-effect level we observed here for growth or for other responses reported elsewhere (Spear and Pierce 1979). The protocol outlined would provide a rapid method for initial estimates of safe levels, based on short-term lethal tests and utilizing a whole-organism quantal response. It should be noted, however, that not all toxicants can be expected to conform to the pattern of response reported here for copper. Although we have noted enhanced tolerance to arsenic in rainbow trout during a 21-d period of acclimation to 0.22 ILL of arsenic, the response to hydrogen cyanide was totally different. Trout acclimated to 0.35 ILL of cyanide were initially sensitized but regained control-level tolerance to cyanide after 3 wk (Dixon and Sprague 1981).

Measurement of changes in tolerance to evaluate toxicant stress could be applied to field situations, although this would encompass genetic as well as physiological adaptation. Louma (1977) suggested that if one population of a species is more tolerant of a toxicant than are other populations, this constitutes strong evidence that the concentration of the toxicant at the site of the more tolerant population was sufficient to elicit biological effects. Similarly, at a site contaminated by more than one toxicant, increased tolerance to some contaminants but not to others would indicate that those to which acclimation was shown were the toxicants of primary concern in that system.

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Abiotic versus Biotic Uptake of Radiophosphorus in Lake Water[1]

STEPHEN J. TARAPCHAK, DAVID R. SLAVENS, AND LISA M. MALONEY[2]

U.S. Department of Commerce, Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration, 2300 Washtenaw Ave., Ann Arbor, MI 48104, USA

TARAPCHAK, S. J., D. R. SLAVENS, AND L. M. MALONEY. 1981. Abiotic versus biotic uptake of radiophosphorus in lake water. Can. J. Fish. Aquat. Sci. 38: 889–895.

Tests were conducted on *Selenastrum capricornutum* and lake water to evaluate the common practice of estimating abiotic uptake of radiophosphorus ($^{33}P_i$) by poisoning samples with glutaraldehyde (GA) and Formalin (FM) and to estimate abiotic uptake in Lake Michigan water. Algae treated with GA and FM release intracellular $^{31}P_i$ into solution, which elevates the dissolved $^{31}P_i$:$^{33}P_i$ ratio and could seriously underestimate abiotic uptake. Carbonylcyanide *m*-chlorophenylhydrazone (CP), an inhibitor of phosphorylation, was identified as a satisfactory agent for estimating abiotic uptake because it effectively inhibits biological uptake, it does not cause the release of detectable amounts of $^{31}P_i$ from algae into solution, and it does not appear to block significantly abiotic uptake by particulate material in lake water. Two types of tests demonstrated that nonbiological uptake of $^{33}P_i$ in water from Lake Michigan was negligible: uptake in samples treated with 10^{-3} mol/L CP was ≤0.01%, and uptake by particulate material recovered from lake water after treatment with heat or GA and resuspension in filtered lake water was ≤2% of rates measured in untreated samples. Procedures for estimating abiotic uptake in $^{33}P_i$ tracer experiments are proposed.

Key words: methodology, carbonylcyanide *m*-chlorophenylhydrazone, Lake Michigan, phosphorus, phytoplankton

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Au moyen de *Selenastrum capricornutum* et d'eau lacustre, on a évalué expérimentalement la façon habituelle d'estimer l'assimilation abiotique de radiophosphore ($^{33}P_i$) par empoisonnement d'échantillons au glutaraldéhyde (GA) et à la Formalin (FM) et on a estimé l'assimilation abiotique dans l'eau du lac Michigan.Les algues traitées au GA et à la FM libèrent du $^{31}P_i$ intracellulaire en solution, ce qui hausse le rapport $^{31}P_i$:$^{33}P_i$ dissous et peut mener à une sous-estimation grossière de l'assimilation abiotique. La *m*-chlorophénylhydrazone carbonylcyanurée (CP), inhibiteur de la phosphorylation, a été identifiée comme un agent permettant d'estimer de façon satisfaisante l'assimilation abiotique parce qu'elle inhibe bien l'assimilation biologique, ne provoque pas la libération de quantités décelables de $^{31}P_i$ des algues en solution, et ne semble pas bloquer de façon significative l'assimilation abiotique par les particules dans l'eau lacustre. Les deux types d'expériences ont permis de démontrer que l'assimilation non biologique de $^{33}P_i$ dans l'eau du lac Michigan était négligeable: l'assimilation dans des échantillons traités avec 10^{-3} mol/L de CP était ≤0,01%, et l'assimilation par les particules prélevés dans l'eau du lac après traitement à la chaleur ou au GA et remise en suspension dans de l'eau lacustre filtrée était ≤2% des taux mesurés dans des échantillons non traités. On propose des méthodes pour estimer l'assimilation abiotique au cours d'expérience où on utilise le $^{33}P_i$.

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UPTAKE of dissolved inorganic PO_4-P ($^{31}P_i$) by phytoplankton usually is measured by adding labeled orthophosphoric acid ($^{32}P_i$ or $^{33}P_i$) to lake water and following its rate of removal by particulate material (e.g. Lean 1973). As $^{33}P_i$ can be sorbed by detrital, silt, or clay particles or can form filter-retainable complexes with iron, calcium carbonate, or humic materials (Olsen 1967), abiotic uptake frequently is estimated in control samples by inactivating biological uptake with heat or poisons (e.g. Rigler 1956; Pomeroy 1963; Levine 1975; Paerl and Lean 1976; Richey 1979). Apparent uptake usually is <5% of that measured in untreated water, suggesting that abiotic

[1]GLERL Contribution No. 224.

[2]Present address: Kalamazoo College, Kalamazoo, MI 49007, USA.

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uptake is negligible. Rigler (1973), however, argues that nonbiological uptake may be substantially underestimated if poisoned microorganisms leak $^{31}P_i$ into solution. Because high specific activity $^{33}P_i$ normally is added to lake water, a release of only 1−2 μg $^{31}P_i$/L from organisms could severely reduce estimates of abiotic uptake as the ratio of $^{31}P_i$ to $^{33}P_i$ in solution would increase dramatically. If abiotic uptake is significantly underestimated, conclusions drawn about the coupling of $^{31}P_i$ supply and utilization rates (Richey 1979) or on the kinetics of $^{31}P_i$ uptake by phytoplankton at low substrate concentrations (Brown et al. 1978) could be seriously biased.

Rigler's hypothesis has never been tested, nor is there a generally accepted poison or procedure for estimating nonbiological uptake. Several studies suggest that poisoned microorganisms leak $^{31}P_i$ into solution and that abiotic uptake may be much higher than previously reported. Silver and Davoll (1978) showed that natural phytoplankton populations release up to 86% of recently assimilated [^{14}C]$NaHCO_3$ 2−72 h after treatment with poisons such as glutaraldehyde (GA) and Formalin (FM). Presumably, $^{31}P_i$ also would be released. Autoradiographic analysis of samples incubated with $^{33}P_i$ suggests that significant amounts of abiotic uptake occur (Fuhs and Canelli 1970; Stull 1972). These techniques may grossly underestimate nonbiological uptake as $^{33}P_i$ can be sorbed by dead or physiologically inactive cells (which would appear as biological uptake), or sorbed $^{33}P_i$ may be lost from particulate material when samples are preserved and then prepared for analysis.

This study was undertaken to identify an inhibitor that inactivates biological activity without causing microorganisms to release $^{31}P_i$, to test Rigler's contention that abiotic uptake can be underestimated if $^{31}P_i$ is released from poisoned microorganisms, and to estimate abiotic uptake in water from Lake Michigan by two independent procedures.

Methods and Materials

Experimental Design

Experiments were designed to evaluate the suitability of various inhibitors of biological uptake and to estimate abiotic uptake of $^{33}P_i$ by particulate material in lake water. GA and FM were chosen as inhibitors because they are used frequently in control samples to inactivate biological uptake. Carbonylcyanide *m*-chlorophenylhydrazone (CP) was selected because it inhibits photophosphorylation in algae and oxidative phosphorylation and $^{31}P_i$ uptake in algae and bacteria (Heyltler and Prichard 1962; Healey 1973a; Kuhl 1974; Macrobbie 1974; Falkowski and Stone 1975; Friedberg 1977). Therefore, CP may be a suitable substance for inactivating biotic uptake in lake water because bacteria, which also can take up $^{33}P_i$ (Paerl and Lean 1976), are inactivated and there is no evidence that the inhibitor causes leakage of $^{31}P_i$ from organisms.

Two conditions must be satisfied to estimate abiotic uptake accurately: a poison must completely inactivate biological uptake without causing leakage of $^{31}P_i$ from microorganisms and the poison itself must not block abiotic uptake. Initially, "model" experiments were performed on laboratory cultures of algae containing large quantities of stored $^{31}P_i$ to determine if the poisons inhibited $^{33}P_i$ uptake or resulted in leakage of $^{31}P_i$ into solution. ^{14}C uptake was measured to determine if CP inhibited photophosphorylation. Cellular release of $^{31}P_i$, coupled with failure of algae to photosynthesize, was accepted as evidence that poisons had killed cells.

Two types of experiments to estimate abiotic uptake in water from Lake Michigan were performed. (1) Tests conducted on cultured algae were repeated using lake water (a) to determine if poisoned microorganisms released substantial amounts of $^{31}P_i$ into solution, (b) to determine if the poisons themselves blocked abiotic uptake, and (c) to test Rigler's (1973) argument that abiotic uptake can be underestimated if $^{31}P_i$ is released from microorganisms. (2) Abiotic uptake also was estimated by performing experiments on biologically inactivated particulate material recovered from samples of lake water. We used heat to kill microorganisms (e.g. Rigler 1956). This is a harsh treatment that may bias estimates by altering surface characteristics of particulate materials or by shifting equilibrium reactions involving chemical complexation of $^{31}P_i$. Therefore, tests also were performed by killing samples with GA, where such effects should be minimal relative to those occurring in heat-killed samples.

Algal Cultures

The green alga *Selenastrum capricornutum* from the Environmental Protection Agency (EPA), Environmental Research Laboratory, Corvallis, OR, was maintained in Guillard's medium (Nichols 1973) ($^{31}P_i$ concentration = 1.55 mg/L) at ~300 lx ("Cool-white" fluorescent bulbs) at 21°C. An inoculum (1−10 mL) from stock cultures in stationary growth was transferred into 800 mL of medium without $^{31}P_i$ in a 1-L Erlenmeyer flask and grown under continuous illumination (~7000 lx) for 3−5 d before experiments. Algae were in an exponential growth phase and hot water extractable $^{31}P_i$ determinations (Fitzgerald and Nelson 1966) showed that cells contained large reserves of stored $^{31}P_i$. The diatom *Stephanodiscus minutus* (obtained from S. Kilham, Univ. of Michigan) and the blue-green alga *Microcystis aeruginosa* (also from the EPA) were used to determine if results obtained with *S. capricornutum* apply to other algae.

Lake Water Samples

Water from Lake Michigan was collected at intervals of 2−3 wk from June to October 1978 and during August and September 1979 from a depth of 4 m with a 20-L PVC Niskin bottle at an inshore (43°04′24″N, 86°16′15″W) and an offshore (43°05′12″N, 86°25′45″W) station, 4 and 15 km, respectively, west of Grand Haven, MI. Samples were transported in insulated jugs to the laboratory in Ann Arbor, MI. Ambient lake water temperatures (±2.0°C) were maintained during shipment. Within 8−12 h, samples were exposed to ~7000 lx in an incubator at lake water temperature for 1−4 h before experiments were performed.

Poisons and Tracer Experiments

CP (obtained from Sigma Chemical Co., St. Louis, MO) was dissolved in 100% ethanol and added to subsamples at

final concentrations ranging from 10^{-6} to 10^{-3} mol/L. (Tests showed that ethanol did not affect photosynthesis or $^{33}P_i$ uptake.) Biological-grade GA (Electron Microscopy Sciences, Fort Washington, PA) and reagent-grade FM were added at final concentrations of 0.25 and 1%, respectively.

In $^{33}P_i$ experiments, 100-mL subsamples were transferred into 250-mL Erlenmeyer flasks and poisons were added. Within 5 s, 37–185 kBq of carrier-free $^{33}P_i$ orthophosphoric acid in 0.02 mol/L HCl (New England Nuclear (NEN), Boston, MA) was added. Five- or 10-mL aliquots were removed within 5 s and at intervals up to 2–6 h and filtered at a vacuum of 39.9 kPa through 0.45-μm HA Millipore filters (25-mm diameter). Filters were assayed in Aquasol (NEN) in a Beckman, model LS-150, liquid scintillation counter (counting errors $< \pm 2\%$). Total $^{33}P_i$ activity was measured by removing unfiltered aliquots and counting them along with filters. The amount of $^{33}P_i$ remaining in the filtrate was calculated by subtracting activity of the filters from total activity (corrected for volume and filter sorption). The rate constant, K (min^{-1}), was computed as the initial slope of the line on a plot of ln $^{33}P_i$ in the filtrate versus time (Lean and Nalewajko 1976). This instantaneous rate constant for $^{31}P_i$ uptake was proportional to relative amounts of $^{33}P_i$ taken up at isotopic equilibrium. Tests showed that precision of K estimation was high (SE = 3.0%, $n = 3$); therefore, treatments in most experiments were not replicated.

Photosynthesis was measured in duplicate 100-mL subsamples in 250-mL Erlenmeyer flasks while $^{33}P_i$ experiments were in progress. Flasks received poisons, followed within 10 s by addition of 111–222 kBq ([^{14}C]$NaHCO_3$ from NEN) and were incubated at ~7000 lx. Duplicate 10-mL aliquots were withdrawn immediately and at 1, 2, and 4 h and filtered at 39.9 kPa through 0.45-μm HA Millipore filters (47-mm diameter). Filters were rinsed with 50 mL of distilled water (lake water samples) or degassed with HCl (algal cultures) to remove inorganic ^{14}C, dried over silica gel, and assayed in the liquid scintillation counter (counting errors $< \pm 5\%$). Uptake was computed by subtracting filter activity at time zero from filter activity of incubated samples.

$^{31}P_i$ concentrations in solution were measured in experiments on algal cultures and in four of the lake water tests. Subsamples were withdrawn immediately before, 10–15 s after, and at intervals up to 4 h after poisons were added and filtered through prerinsed 0.45-μm HA Millipore filters (47-mm diameter) at 39.9 kPa. $^{31}P_i$ was measured by acid molybdate/stannous chloride reduction using internal standards containing poisons (detection limit 0.8 μg P/L; precision at 5.0 μg P/L = ±0.08 μg P/L) (Environment Canada 1974). Subsamples treated with CP also were analyzed by Chamberlain and Shapiro's (1969) extraction technique (detection limit 0.3 μg P/L) to determine if leakage of $^{31}P_i$ could be detected. Additional tests showed that GA, CP, and FM did not introduce contaminant $^{31}P_i$ into subsamples. The pH of lake water samples (ranging between 8.0 and 8.3) was not affected by CP. GA and FM lowered pH in the range of 7.3 to 7.7 and 7.5 to 7.9, respectively.

To ensure that microorganisms were biologically active, subsamples of lake water and cultured algae were spiked with 10 and 25 μg $^{31}P_i$/L (as KH_2PO_4), respectively. If $^{33}P_i$ is being taken up and utilized biologically, $^{31}P_i$ should be removed from solution, ^{14}C uptake should be stimulated, and K should decrease because of the increase in the $^{31}P_i$ to $^{33}P_i$ ratio (Healey 1973a, b; Rigler 1973).

Abiotic Uptake by Particulate Material

Four experiments in 1979 were designed to determine if CP inhibited abiotic uptake, to test Rigler's contention, and to provide independent estimates of abiotic uptake. Particulate material was obtained by killing samples of lake water and recovering and resuspending particulate material in filtered lake water and in distilled water (pH 6.0–7.0). Particulate material in distilled water should have a high capacity for rapid $^{33}P_i$ uptake because $^{31}P_i$ undoubtedly is released from particulates (increasing the number of available uptake sites) (i.e. a strong concentration gradient is set up between particulate material and distilled water) and because removal of $^{31}P_i$ from solution decreases the $^{31}P_i$:$^{33}P_i$ ratio. These subsamples provide suitable material to determine if CP inhibits abiotic uptake and to test the potential importance of $^{31}P_i$ release from microorganisms on abiotic uptake rates. Particulate materials recovered in filtered lake water will give estimates more representative of abiotic uptake in fresh, untreated lake water as the original $^{31}P_i$ concentration is maintained throughout sample preparation (see below).

Samples were prepared as follows. Two 500-mL (or 1-L) samples of fresh lake water were heated to 85°C for 15 min, cooled, and poured into two sets of centrifuge tubes and centrifuged at 1250 rpm for 15 min. Supernatant from the tubes was discarded; one set of tubes was filled with filtered lake water and the other with distilled, deionized water. Distilled water and lake water were filtered through 0.10-μm membrane filters to remove abiotic particles or biological contaminants that could remove $^{33}P_i$ from solution. The washing and centriguation procedure was repeated three times. Particulate material in each set of tubes was recovered in 500-mL (or 1-L) volumetric flasks; one flask was diluted with filtered lake water and the other with distilled, deionized water. Flasks were shaken to ensure uniform distribution of particles and 100-mL subsamples were transferred into 250-mL Erlenmeyer flasks. Tests showed that treatment with heat and GA had inactivated microbial activity. [^{14}C]$NaHCO_3$ and [^{14}C]glucose uptake by particulate material could not be detected, indicating that $^{33}P_i$ uptake was due to abiotic precesses. Subsamples of fresh lake water were included to measure combined biotic and abiotic uptake. Isotope additions, filtrations, and assay of filters in 3-h experiments were performed as outlined above.

Glassware used in all phases of the investigation was washed in hot water and Liquinox (Alconox, Inc., New York), a phosphate-free detergent, rinsed with tap water, soaked in concentrated HNO_3 for at least 8 h, rinsed six times in distilled, deionized water.

Results and Discussion

Effects of Poisons on Microorganisms

Algal Cultures — Selenastrum capricornutum responded similarly to additions of CP, GA, and FM in four replicate

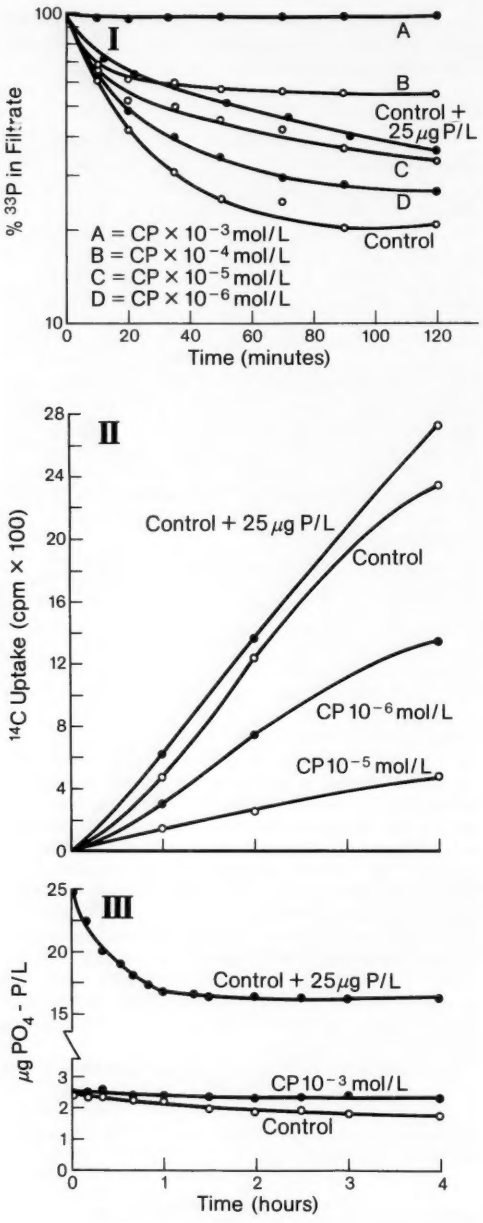

FIG. 1. Loss of $^{33}P_i$ from solution (I), photosynthetic ^{14}C uptake (II), and $^{31}P_i$ release (III) from *Selenastrum capricornutum* treated with $10^{-6}-10^{-3}$ mol/L CP, 0.25% GA, and 1% FM. Uptake of $^{33}P_i$ in the presence of GA and FM was <0.10%. Uptake of ^{14}C at 10^{-4} and 10^{-3} mol/L CP and in subsamples treated with GA and FM could not be detected. Concentrations of $^{31}P_i$ (III) at 10^{-6}, 10^{-5}, and 10^{-4} mol/L CP were intermediate between those measured at 10^{-3} mol/L CP and the control.

experiments and in the predicted way to a spike of $^{31}P_i$ (cf. Fig. 1). CP effects were concentration dependent: $^{33}P_i$ and ^{14}C uptake were blocked at 10^{-3} mol/L. Small amounts (<2.5%) of $^{33}P_i$ taken up at 10^{-3} mol/L CP, consistently detected during the first 30 min, indicate that time was required to block uptake completely. CP did not cause a detectable release (>0.3 μg P/L) of intracellular $^{31}P_i$ into solution. Treatment with GA and FM killed algae and resulted in large, rapid releases of $^{31}P_i$ into solution (Fig. 2). The source of this $^{31}P_i$ is probably from acid hydrolysis of polyphosphate, the principal storage product of most algae (Healey 1973b). Experiments using *S. minutus* and *M. aeruginosa* gave the same type of results, including large releases of $^{31}P_i$ into solution when cultures were treated with GA and FM.

Lake Water — Results from one series of experiments are shown in Fig. 3 and data from all tests are summarized in Table 1. Microorganisms assimilated added $^{31}P_i$, as shown by its loss from solution, by an increase in photosynthesis and by a decrease in *K* (not shown on graph). Uptake of $^{33}P_i$ and ^{14}C was blocked by 10^{-4} or 10^{-3} mol/L CP and releases of $^{31}P_i$ into solution could not be detected. GA and FM killed microorganisms, releasing $^{31}P_i$ in quantities 1.5−2.9 times the original concentration in the water. Small amounts (<0.12%) of $^{33}P_i$ uptake detected in several tests were the result of minute amounts of abiotic uptake as corrections for $^{33}P_i$ sorption onto filters had been made. $^{31}P_i$ may have been released from abiotic sources as GA and FM decreased the pH of lake water (see Methods and Materials). To test this the pH of several samples was lowered to pH 6.0−7.0 with HCl. Elevations in $^{31}P_i$ could not be detected, so release from microorganisms must have been the source.

These results suggest that CP is a suitable substance for estimating nonbiological $^{33}P_i$ uptake, provided that it does not block abiotic uptake. Comparisons of the effects of 10^{-5} mol/L CP on uptake by particulate material in distilled water subsamples showed that it strongly inhibited $^{33}P_i$ uptake (Table 2). In subsamples where particulate material had been washed and resuspended in filtered lake water, however, CP either had no effect or reduced uptake by a small amount. The inhibitor, therefore, did not block abiotic uptake significantly in untreated samples of water (see below).

Abiotic Uptake in Lake Michigan Water

Test of Rigler's Hypothesis — Rigler's (1973) contention that abiotic uptake could be underestimated if poisoned microorganisms leak $^{31}P_i$ into solution was tested in several experiments. Tests were performed by spiking split subsamples of particulate material with 3.0 μg P/L (KH_2PO_4). Uptake of $^{33}P_i$

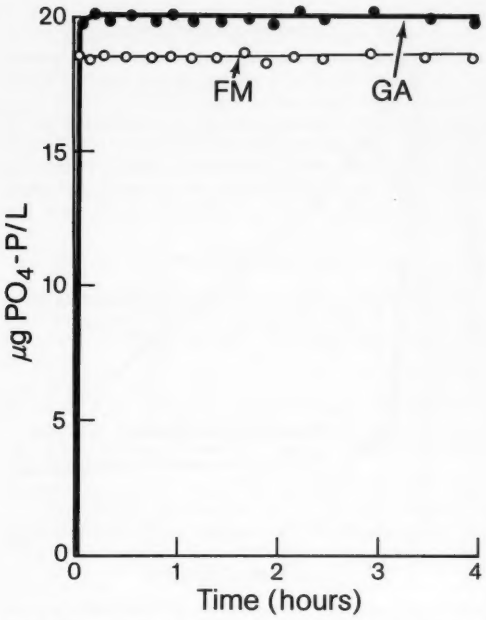

FIG. 2. $^{31}P_i$ release from *Selenastrum capricornutum* treated with 0.25% GA and 1% FM.

in distilled water treatments was reduced by two orders of magnitude relative to unspiked subsamples, indicating that abiotic uptake potentially can be severely underestimated by relatively small releases of $^{31}P_i$ from microorganisms (Table 2). Under conditions more representative of uptake by particulate materials in Lake Michigan water (i.e. lake water treatments), $^{31}P_i$ spikes decreased K by much smaller amounts. The release of $^{31}P_i$ into solution (Table 1), caused by leakage of $^{31}P_i$ from poisoned microorganisms, did not seriously bias estimates as the rates were very low. In lakes where abiotic uptake may be higher, however, small releases of $^{31}P_i$ into solution could cause nonbiological uptake to be seriously underestimated (see below).

Estimates of Abiotic Uptake — The relative importance of abiotic uptake also was evaluated by comparing uptake rates by particulate material recovered from heat-killed samples of water relative to those in untreated samples of water. Results from one test (Fig. 4) and data from three other experiments showed that, although the potential for abiotic uptake was significant (8–32% of controles in distilled water treatments), K was very low (<2.0% of controls) in subsamples washed and resuspended in filtered lake water (Table 2). These are maximal estimates because microbial biomass has been converted into abiotic particulate material, and living microorganisms are not competing with abiotic processes for $^{33}P_i$.

Tests also showed that heat treatment did not bias estimates. Samples killed with 0.25% GA and washed and

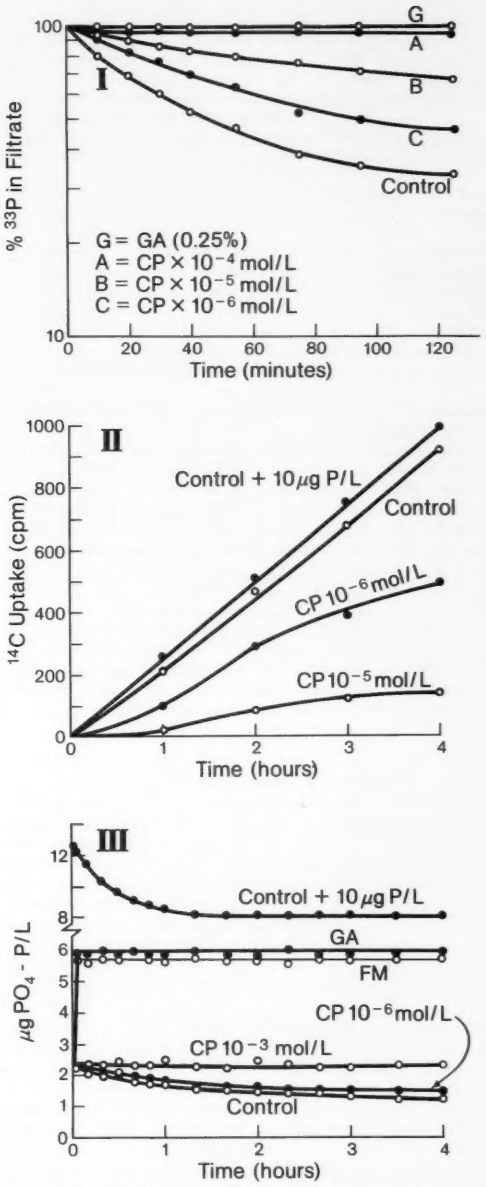

FIG. 3. Loss of $^{33}P_i$ from solution (I), photosynthetic ^{14}C uptake (II), and $^{31}P_i$ release (III) from microorganisms in water collected on October 2, 1978, at an offshore station in Lake Michigan treated with 10^{-6}–10^{-3} mol/L CP, 0.25% GA, and 1% FM. Uptake of $^{33}P_i$ was <0.10% in treatments of 10^{-3} mol/L CP and 1% FM. ^{14}C uptake at 10^{-3} and 10^{-4} mol/L CP, 0.25% GA, and 1% FM could not be detected. Concentrations of $^{31}P_i$ (III) at 10^{-5} mol/L CP.

TABLE 1. Effects of $10^{-6}-10^{-3}$ mol/L CP, 0.25% GA, and 1% FM on $^{33}P_i$ uptake (K, in min^{-1}), ^{14}C uptake (average $cpm \cdot min^{-1}$ for the 4-h incubation period), and release of $^{31}P_i$ in water from Lake Michigan.

Treatment	Overall mean as percent of control	Overall range as percent of control
	$^{33}P_i$ uptake (K) (min^{-1})	
Control	100	100–100
CP (10^{-6} mol/L)	63.1	59–68
CP (10^{-5} mol/L)	32.2	30–40
CP (10^{-4} mol/L)	6.30	4.0–9.0
CP (10^{-3} mol/L)	0.009	0.008–0.01
GA (0.25%)	0.09	0.00–0.12
FM (1%)	0.007	0.00–0.10
	^{14}C uptake ($cpm \cdot min^{-1}$)	
Control	100	100–100
CP (10^{-6} mol/L)	54.4	49–63
CP (10^{-5} mol/L)	14.8	9–17
CP (10^{-4} mol/L)	1.82	0.00–2.0
CP (10^{-3} mol/L)	0.00	0.00–0.00
GA (0.25%)	0.00	0.00–0.00
FM (1%)	0.00	0.00–0.00

	No. of tests			
	1	2	3	4
Release of $^{31}P_i$ (μg $^{31}P_i$/L released into solution)				
GA (0.25%)	6.0	2.9	2.5	3.7
FM (1.0%)	5.7	2.8	2.6	3.4

TABLE 2. Comparison of $^{33}P_i$ uptake (K, in min^{-1}) by particulate material in heat-killed subsamples of lake water (abiotic uptake) washed and resuspended in filtered lake water and in distilled water, and by untreated subsamples (biological plus abiotic uptake) in water collected at an inshore (September 11, 1979) and an offshore (August 20 and 29, 1979) station in Lake Michigan. Blockage of uptake by 10^{-5} mol/L CP and the effects of a $^{31}P_i$ spike also were measured.

Treatment	$^{33}P_i$ uptake (K) (min^{-1})		
	Aug. 20 1979	Aug. 29 1979	Sept. 11 1979
Lake water	0.0012	0.0054	0.0019
Lake water + 3 μg $^{31}P_i$/L	0.0002	0.0004	0.0003
Lake water + 10^{-5} mol/L CP	0.0007	0.0033	0.0019
Distilled water	0.0288	0.0307	0.0173
Distilled water + 10^{-5} mol/L CP	0.0041	0.0038	0.0019
Distilled water + 3 μg $^{31}P_i$/L	0.0005	0.0009	0.0007
Control	0.0901	0.2983	0.2211

recovered in filtered lake water on August 20, August 29, and September 29 gave K values of 0.0009, 0.0052, and 0.0020 min^{-1}, respectively. We conclude, therefore, that nonbiological uptake of $^{33}P_i$ in fresh, untreated samples of lake water was negligible and comparable to values obtained by using 10^{-3} mol/L CP.

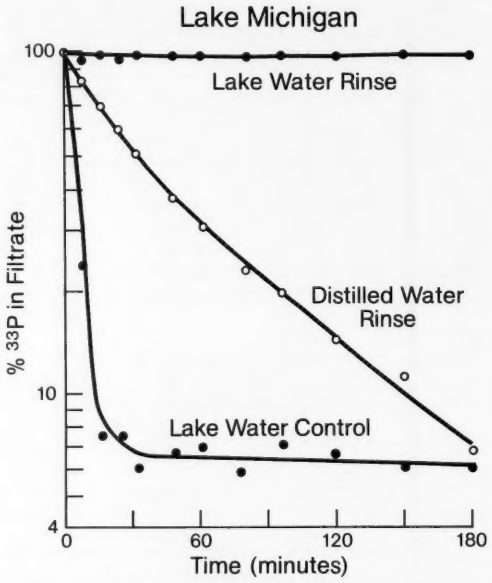

FIG. 4. Comparison of $^{33}P_i$ uptake by particulate material in an untreated subsample of lake water and by particulate material recovered from heat-killed subsamples that were washed, centrifuged, and resuspended in filtered lake water and in distilled water. Sample was collected August 8, 1979, from an offshore station in Lake Michigan.

Summary and Conclusions

Two arguments force the conclusion that abiotic uptake of $^{33}P_i$ in water from Lake Michigan is almost exclusively biotic. (1) Uptake of $^{33}P_i$ is negligible ($\leq 0.01\%$) in the presence of 10^{-3} mol/L CP, and $^{31}P_i$ release from microorganisms, which would cause abiotic uptake to be underestimated, was not detected. (2) Insignificant amounts of $^{33}P_i$ were taken up by particulate material recovered from heat- or GA-treated samples resuspended in filtered lake water. This conclusion is applicable to Lake Michigan, where epilimnetic water is moderately buffered (total alkalinity ranges between 104 and 115 mg/L, phenolphthalein alkalinity is <5.0 mg/L, pH varies between 7.9 and 8.5) and phytoplankton are limited by phosphorus from July through October (S. J. Tarapchak, Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration, Ann Arbor, MI, unpublished data). Abiotic uptake, however, may be very important in lakes with high silt or clay loadings (Olsen 1967; Golterman 1973) or in waters with high concentrations of iron, calcium, or humic materials (Olsen 1967; Otsuki and Wetzel 1972; Koenings and Hooper 1976; Francho and Heath 1979), where rapid chemical complexation by particulates may compete with biotic uptake for $^{33}P_i$.

Rigler's contention that abiotic uptake may be underestimated if $^{31}P_i$ is released from poisoned microorganisms does not apply to Lake Michigan but it is a generally warranted concern (Table 2). Although CP potentially can block

abiotic uptake (Table 2), it is a more suitable agent than FM or GA for estimating nonbiological uptake in tracer experiments. We recommend that CP be used to estimate abiotic uptake in lakes where loadings of silt, clay particules, or organic materials are low and where chemical complexation of $^{31}P_i$ is minimal. In other environments, estimates can be obtained by killing samples with heat or GA, suspending particulate material in filtered lake water, and measuring $^{33}P_i$ uptake relative to rates in untreated lake water. These values will be the highest possible for abiotic uptake in lake water.

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Patterns of Invertebrate Size and Abundance in Aquatic Habitats

GARY G. MITTELBACH[1]

W. K. Kellogg Biological Station and Department of Zoology, Michigan State University, Hickory Corners, MI 49060, USA

MITTELBACH, G. G. 1981. Patterns of invertebrate size and abundance in aquatic habitats. Can. J. Fish. Aquat. Sci. 38: 896–904.

The size distributions of invertebrate prey found in the vegetation, bare sediment, and open water habitats of a small, Michigan lake were quantified for the months of May through August, 1979. The distribution of prey body size in each habitat generally conformed to a lognormal distribution, allowing a simple characterization of prey size and abundance in each habitat. Seasonally, the abundance of large littoral invertebrates declined markedly, shifting the distribution of prey size in the vegetation and bare sediment habitats towards smaller prey. The total biomass of vegetation-dwelling prey (exclusive of gastropods) also declined dramatically from May to August. These seasonal changes in prey size and abundance were correlated with a reduction in foraging intake for the dominant fish species in the lake, the bluegill sunfish (*Lepomis macrochirus*). Bluegills whose diet was >80% vegetation prey showed a >3-fold decline in stomach contents (prey weight/fish weight) from May to Ausut, during which time prey biomass in the vegetation declined 2.5-fold.

Key words: body size, lognormal distribution, benthos, foraging, *Lepomis*, Michigan

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Entre mai et août 1979 inclusivement, nous avons quantifié la distribution des tailles d'invertébrés proies habitant la végétation, le sédiment nu et la grande eau d'un petit lac du Michigan. La distribution de la grosseur du corps des proies dans chaque habitat est généralement long-normale, de sorte qu'une simple caractérisation de la taille et de l'abondance des proies est possible pour chaque habitat. L'abondance des grands invertébrés du littoral dimiune notablement avec la saison, produisant un glissement vers une taille plus petite de la distribution de la taille des proies habitant la végétation et le sédiment nu. La biomasse totale des proies vivant parmi la végétation (à l'exclusion des gastéropodes) diminue de façon dramatique, elle aussi, entre mai et août. Nous avons établi une corrélation entre ces changements saisonniers de la taille et de l'abondance des proies d'une part et une réduction de prédation par l'espèce dominante de poisson dans le lac, le crapet arlequin (*Lepomis macrochirus*) d'autre part. Entre mai et août, il se produisit chez les crapets arlequins dont plus de 80 % du régime était constitué de proies vivant dans la végétation une diminution de plus de 3 fois du contenu stomacal (poids des proies/poids du poisson), alors que, dans cette même période, la biomasse des proies dans la végétation diminuait de 2,5 fois.

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THE relative size of predator and prey is of major importance in determining a fish's diet, energetic intake, and rate of growth. Recent models of prey selection stress the components of predation (c.f. Holling 1959), among the most important of which are the rate at which a predator encounters prey and the time required to handle a prey item. In fishes, both of these components are directly related to prey size (Ware 1972; Werner 1974; Werner and Hall 1974; Eggers 1977; Mittelbach 1981), and feeding models which incorporate prey encounter rates, handling times, and energetic contents as functions of prey size have shown considerable success in predicting diet selection (Werner and Hall 1974; Mittelbach 1981; Werner and Mittelbach 1981). Feeding efficiency and growth in fishes is also strongly dependent on available prey size (Paloheimo and Dickie 1966; Wankowski and Thorpe 1979), and considerable evidence indicates that the availability of large prey items in the environment is crucial for the sustained growth of most fishes (Martin 1970; Kerr 1971; Mittelbach 1980). Given the importance of prey size to the foraging behavior and growth of fishes, it seems crucial that fisheries biologists and ecologists have detailed descriptions of the distribution and abundance of prey sizes in natural habitats.

Towards this end I have quantified the size distributions of invertebrate prey found in the vegetation, bare sediment, and open water habitats of a small marl lake in southwest Michigan during the months of May through August, 1979. These three habitat types represent important divisions of the

[1]Present address: Department of Zoology, Ohio State University, Columbus, OH 43210, USA.

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natural environment for many fish species as each habitat differs markedly in physical structure and associated prey species. Consequently, each habitat requires unique modes of foraging by fish. Previous work has demonstrated that such natural prey size distributions, when placed within relevant foraging theory, can be extremely useful in the study of competitive interactions (Wilson 1975; Werner 1977), habitat selection (Mittelbach 1981), and "optimal" body size relations in predators (Schoener 1969). This study extends our knowledge of the distribution of preys sizes in aquatic systems by examining the seasonal dynamics of prey sizes in three habitats in a natural lake.

Methods

Site

Lawrence Lake, a mesotrophic marl lake, 4.9 ha in surface area, located 2.1 km east of Hickory Corners, Barry County, MI, was selected for study as it contains a very simple and stable plant community (79% *Scirpus subterminalis* by weight (Rich et al. 1971)) and a number of areas of discrete habitat types, and receives only slight fishing pressure. The east shore of Lawrence Lake was used as the primary study site. Here the bench is characterized by areas of barren marl sediments (85% open bottom) extending 8–12 m from shore to an average depth of 0.75 m. The far edge of the bench and the entire slope region, which drops steeply from 1.5 to 6 m, are covered by a dense stand of *S. subterminalis* (82% cover), 0.4–0.6 m in height. Beyond the littoral zone the lake bottom slopes gradually to a maximum depth of 12.6 m located ~100 m from the east shore.

Prey Sampling

Invertebrate prey were sampled from each of three habitats (barren marl bench, vegetated bench and slope, and open water) during the months of May–August, 1979. Estimating the total distribution of prey sizes in each habitat presented a problem with regard to choosing the appropriate sample size because small prey were likely to be extremely abundant and large individuals rare (Schoener and Janzen 1968; Werner 1977). In fact, prey abundances, by 1-mm size-classes, ranged over four orders of magnitude in both the vegetation and sediment habitats. Because the number of samples required to estimate benthic population numbers (at a given level of precision) increases dramatically with a decline in population number (Downing 1979; Resh 1979), it is clear that the very large number of samples required to sample rare size-classes accurately would be completely inappropriate for most prey sizes present. Sample sizes used were therefore chosen as a compromise between accurately estimating the abundance of rare large individuals and avoiding handling huge numbers of the small size-classes.

Prey living in the marl sediments were sampled with a Plexiglas corer (6.5 cm in diameter; 8 cm deep) by a diver using mask and snorkel or SCUBA. The diver's corer is the most successful sampling device for this type of habitat (Flannagan 1970). Five random samples were taken on each sampling date. After collection, samples were removed to the laboratory and gently washed through a series of U.S. Standard sieves (2.83, 0.710, 0.180 mm). Organisms retained by the two larger meshed sieves were removed by hand and preserved in 10% Formalin. Contents of the smallest meshed sieve were mixed with a 20% solution of $MgSO_4$ which allowed separation of live prey from inorganic sediments and debris.

The vegetation habitat was sampled by a diver using a modified version of Gerking's sampler (Gerking 1957). Basically, the device consisted of a Plexiglas cylinder (21.5 cm in diameter) with a No. 10 plankton net and quart Mason jar attached at the top. At the base of the cylinder were two sliding plates which could be closed to form a seal. A sample was then taken by opening the plates, slipping the cylinder completely over the vegetation, and rapidly sliding the plates closed. As the dominant vegetation encountered (*S. subterminalis*) was quite thin and flexible, the closed plates formed a tight seal around the base of the vegetation. The sample was then removed to the surface by either uprooting the vegetation or cutting it at the base with a knife. At the surface the sampler was inverted and the vegetation and associated prey washed from the plankton net into the attached jar (or into a large bucket if necessary). This sampler is quite effective in capturing prey from the size of small cladocerans to large motile prey such as damselflies and mayflies. Sampling was stratified with five samples taken randomly along the bench (0.5–1.5 m in depth) and five samples taken randomly along the slope (2–4 m in depth) on each date. After collection, the vegetation samples were carefully washed into a series of sieves (2.83, 0.710, 0.180 mm) and treated the same as the sediment samples.

Open water prey were sampled using a 30-cm-diameter No. 10 plankton net. Two vertical tows from a depth of 4 m were taken on each date at a location in the limnetic zone ~60 m from shore. A depth of 4 m was chosen as it defines the usual depth of the thermocline in Lawrence Lake during the summer (Wetzel 1975). Previous work has suggested that the warm water fish of these small lakes remain above the thermocline when feeding (Hall et al. 1979), and echosounder traces taken in Lawrence Lake on July 29, 1979, confirmed that all fish were located at depths less than 4–5 m (thermocline at 4 m (R. G. Wetzel, Kellogg Biological Station, Hickory Corners, MI, personal communication)). Plankton samples were subsequently preserved in 95% alcohol.

Resource sampling was conducted during the hours immediately after dawn as many fish species exhibit a feeding peak during these hours (Keast and Welsh 1968; Sarker 1977; Wilsmann 1979). On average, plankton sampling began 15 min before sunrise and was completed by 10 min after sunrise. Vegetation and sediment sampling began on average 30 min after sunrise and was completed by 70 min after sunrise. Resources sampled thus represent prey availabilities during this morning feeding period.

Prey samples were enumerated under a dissection microscope at 25× and organisms classified, generally to genus or family level. Large vegetation- and sediment-dwelling prey were counted in their entirety. Prey collected on the smallest sieve and the open water plankton were subsampled by placing the sample in a scored petri dish and counting the number of prey organisms in 4 of 16 divisions chosen at random. Fifteen to 50 randomly chosen individuals in each prey category were measured for total body length in each

sample using an ocular micrometer. Prey size–frequency distributions were then constructed for each sample by grouping prey into 0.5 mm classes for the vegetation and sediment habitats and into 0.1-mm classes for the open water plankton. The overall size–frequency distribution of prey available in a habitat was determined by averaging across all samples from a given date. Length–weight regressions were also obtained for each prey taxon permitting the conversion of prey lengths to dry weights (exclusive of shells or cases).

Results

Body Size Distributions

The distributions of prey size available in the vegetation, sediments, and open water show a strong numerical dominance by small prey (Tables 1–3), with the range of prey sizes being greatest in the vegetation and least in the open water. Such distributions are generally lognormal (Aitchison

TABLE. 1. Size distribution of invertebrate prey in the vegetation of Lawrence Lake ($\bar{x}$ ± 1 SE); n = 10 samples per date.

Prey length (mm)	Prey density (no./m^3)			
	May 21	June 14	July 19	Aug. 23
0–0.5	31506±7976	62210±7976	14552±4399	7416±770
0.5–1.0	29853±3147	31357±4076	30735±4881	17246±2319
1.0–1.5	6155±644	12357±1934	16029±2970	7499±1441
1.5–2.0	1813±188	2353±290	10860±2237	2408±508
2.0–2.5	3835±1048	1938±238	4766±853	1741±362
2.5–3.0	3069±1197	1691±340	1691±337	909±253
3.0–3.5	2937±1401	1229±302	1901±383	419±106
3.5–4.0	920±214	672±154	446±111	164±60
4.0–5.0	1146±232	584±129	799±143	11±11
5.0–6.0	419±96	181±59	391±124	0
6.0–7.0	242±66	94±28	187±39	0
7.0–8.0	308±63	44±16	171±65	0
8.0–9.0	231±48	50±17	44±18	5±5
9.0–10.0	105±28	44±21	55±33	0
10.0–12.0	171±52	22±12	11±7	0
12.0–14.0	55±16	38±27	5±5	0
14.0–16.0	11±7	5±5	0	0
16.0–18.0	5±5	5±5	5±5	0
18.0–20.0	5±5	0	5±5	0
Mean length (± 1 SE)	1.07±0.02	0.72±0.02	1.33±0.17	1.07±0.02

TABLE. 2. Size distribution of invertebrate prey in the bare sediments of Lawrence Lake ($\bar{x}$ ± 1 SE); n = 5 samples per date.

Prey length (mm)	Prey density (no./m^3)			
	May 21	June 14	July 19	Aug. 23
0–0.5	3762±1686	2262±924	12037±4509	8337±1436
0.5–1.0	18062±7644	18062±6969	54938±12215	48525±3535
1.0–1.5	3762±1686	21075±4222	82025±7369	83375±19809
1.5–2.0	15050±4122	12037±2760	54175±4222	47712±8431
2.0–2.5	28600±7294	21075±5895	42887±1911	18800±4809
2.5–3.0	30100±6170	17312±6570	16550±2798	8350±1536
3.0–3.5	17312±3872	7525±3947	4512±1836	4125±1424
3.5–4.0	4512±3010	1500±924	4512±2760	3250±1998
4.0–5.0	5637±1499	5262±1911	5187±1386	4662±2823
5.0–6.0	3075±1461	1500±924	2887±1973	0
6.0–7.0	750±750	750±750	750±750	0
7.0–8.0	750±750	0	750±750	750±750
8.0–9.0	0	0	0	0
9.0–10.0	0	0	0	0
10.0–12.0	750±750	0	0	1250±862
Mean length (± 1 SE)	2.51±0.12	2.04±0.09	1.64±0.05	1.55±0.07

TABLE. 3. Average size distribution of invertebrate prey in the open water of Lawrence Lake ($x \pm 1$ SE); calculated from eight samples taken between May and August, 1979.

Prey length (mm)	Prey density (no./m^3)
0–0.1	0
0.1–0.2	80±80
0.2–0.3	3260±1460
0.3–0.4	7920±2810
0.4–0.5	2530±1180
0.5–0.6	2140±680
0.6–0.7	3060±880
0.7–0.8	2730±830
0.8–0.9	1410±350
0.9–1.0	690±290
1.0–1.1	1330±390
1.1–1.2	430±160
1.2–1.3	210±120
1.3–1.4	60±30
1.4–1.5	90±40
1.5–1.6	30±10
1.6–1.7	40±20
1.7–1.8	10±10
1.8–1.9	10±10
>1.9	<10

and Brown 1957). Using a computer program developed by Gauch and Chase (1974) normal curves were fitted to logarithmic transforms of prey lengths in each habitat; the fitted parameters of the normal distributions are listed in Table 4. As the method of Gauch and Chase (1974) is a regression approach, one can examine the proportion of the total variance explained by fitting the lognormal distribution, R^2, to determine the goodness of fit. Examination of the R^2 values in Table 4 indicates that the distribution of prey sizes in each habitat was quite adequately described by the lognormal curve as the fitted distribution generally accounted for greater than 90% of the variance. Because prey weight (W) can be related to length (L) by a power function such as $L = cW^s$, this implies that prey weights are also distributed lognormally in each habitat (Aitchison and Brown 1957; Schoener and Janzen 1968).

The range of prey sizes available in the three habitats is reflected in the variances of the fitted distributions, the standard deviation being greatest in the vegetation and least in the plankton (Table 4). These differences in variances between habitats result in large differences in the relative biomass of small and large prey and can have marked effects on the feeding preferences of fish (see Mittelbach 1981).

SEASONAL PATTERNS

There was a marked reduction in the abundance of large prey in the vegetation habitat across the summer (Table 1). Prey ≥3.0 mm body length declined significantly from 6540/m^3 in May to 2965/m^3 in June, 3938/m^3 in July, and 1408/m^3 in August (ANOVA, $P < 0.01$). The loss of larger instars of Ephemeroptera, Tricoptera, Odonata, and *Hyalella* from the vegetation accounted for most of this reduction.

TABLE. 4. Parameters of the normal distribution fitted to log e of prey length in three habitats. R^2 equals the proportion of the total variance accounted for by the fitted curve.

	Mean prey size (mm)	Standard deviation	Density at the mean (no./m^3)	R^2
		Vegetation		
May 21	0.21	1.115	34658	0.90
June 14	0.28	0.817	63059	0.99
July 19	0.59	0.678	32357	0.99
Aug. 23	0.60	0.586	19808	0.99
Seasonal avg.	0.18	1.250	32794	0.91
		Sediments		
May 21	2.41	0.322	32637	0.68
June 14	1.20	0.688	21162	0.82
July 19	1.16	0.518	82000	0.97
Aug. 23	1.14	0.421	86512	0.98
Seasonal avg.	1.13	0.622	45900	0.98
		Plankton		
Seasonal avg.	0.36	0.487	5240	0.76

Keast (1977) also reports the progressive loss of larger individuals of Ephemeroptera, Odonata, and Isopoda over this time period in Lake Opinicon, Ont. This shift in the vegetation prey size distribution towards smaller prey is evident in the steady decrease in the standard deviation of the fitted lognormal curve from May to August (Table 4).

The total biomass of insect prey also declined from May to August, as did the biomass of amphipods and ostracods (Table 5) (ANOVA: total insects, $P < 0.02$; amphipods $P < 0.06$; ostracods, $P < 0.01$). Together these three prey groups averaged 45% of the total prey biomass in the vegetation across the summer. The seasonal decline in these prey was largely offset by the increasing biomass of gastropods, so that the total biomass of all prey in the vegetation showed little change from May to July (Table 5). A sharp reduction in total biomass did occur in August when gastropods declined about 30% (Tukey's test, $P < 0.01$).

Because most fish species do not possess the specialized adaptations required to feed effectively on snails (e.g. a crushing pharyngeal "mill" with large molariform teeth (Keast 1978; Laughlin and and Werner 1980)), it is informative to examine the patterns in total prey biomass exclusive of gastropods. Table 6 shows that the total biomass of nongastropod prey declined steadily and by more than 2.5-fold from May to August (ANOVA, $P < 0.02$). Similar reductions in the total volume of prey over this time period have been reported by Ball and Hayne (1952) and Anderson and Hooper (1956). Combined, the seasonal decline in prey biomass (exclusive of gastropods) and the decreasing abundance of larger prey dramatically reduce the potential foraging return available to fish in the vegetation over the summer (Mittelbach 1981).

Comparisons between different depth regions in the vegetation (Table 6) indicate that both prey biomass and prey size were larger on the bench (0.5–1.5 m deep) than on the slope (2–4 m deep); (ANOVA, biomass per metre cubed: nongastropod prey, $P < 0.01$; all prey, $P < 0.01$; ANOVA, mean prey size (milligrams): nongastropod prey, $P < 0.01$;

TABLE. 5. Mean number (and dry weight) per metre cubed of major prey groups found in the vegetation (10 samples per date). Total prey numbers, total prey biomass, and mean prey weights are ±1 SE.

	Vegetation prey no./m³ (mg dry wt/m³)			
	May 21	June 14	July 19	Aug. 23
Simocephalus	4361 (88)	4950 (211)	2271 (38)	2068 (33)
Sida	236 (10)	357 (12)	10109 (383)	3366 (71)
Bosmina	4224 (6)	33814 (37)	951 (1)	0
Chydoridae	22817 (66)	31930 (441)	6281 (53)	4276 (33)
Total Cladocera	31638 (170)	70993 (411)	19601 (482)	9702 (142)
Copepoda	21340 (81)	23067 (126)	22302 (92)	11599 (32)
Ostracoda	11637 (257)	6050 (105)	4634 (82)	1603 (60)
Hyalella	2728 (235)	3399 (132)	3465 (166)	1958 (49)
Hydracarina	1133 (86)	1743 (138)	1633 (135)	1243 (87)
Chironomidae	6750 (372)	5345 (140)	21180 (363)	5422 (140)
Other Diptera	4759 (108)	1532 (27)	1598 (83)	898 (4)
Tricoptera	473 (293)	154 (42)	451 (37)	390 (25)
Ephemeroptera	1254 (595)	1270 (285)	2100 (123)	1704 (82)
Odonata	192 (228)	121 (248)	220 (45)	231 (276)
Total Insecta	13293 (1534)	8448 (747)	22907 (653)	8811 (535)
Gastropoda	562 (265)	804 (1279)	1174 (1230)	1003 (694)
Total no.	82687±7612	114669±9113	82557±11544	38593±5211
Total biomass	(2639±451)	(2946±492)	(2856±587)	(1603±145)
Mean prey wt (mg)	0.032±0.006	0.026±0.005	0.034±0.006	0.041±0.008

TABLE. 6. Total dry weight (mg/m³) and mean size (mg dry wt) of prey organisms found in the bench and slope regions of the vegetation. Total weights are $x \pm 1$ SE based on five samples per date for both the bench and slope. Prey sizes are $x \pm 1$ SE based on >600 measurements per date.

	May 21	June 14	July 19	Aug. 23
	Prey dry weight (mg/m³)			
Bench				
All prey	3729±534	4033±680	3929±939	1816±185
No gastropods	3395±580	1910±431	1942±244	1463±215
Slope				
All prey	1548±190	1860±203	1783±308	1390±195
No gastropods	1351±146	1432±219	1310±256	356±49
Combined				
All prey	2639±451	2946±492	2856±587	1603±145
No gastropods	2374±422	1667±241	1626±197	910±211
	Mean prey size (mg dry wt)			
Bench				
All prey	0.052±0.013	0.037±0.006	0.040±0.008	0.039±0.005
No gastropods	0.047±0.013	0.017±0.004	0.021±0.001	0.033±0.007
Slope				
All prey	0.018±0.003	0.016±0.002	0.031±0.009	0.049±0.002
No gastropods	0.017±0.002	0.013±0.002	0.023±0.006	0.013±0.001
Combined				
All prey	0.032±0.006	0.026±0.005	0.034±0.006	0.041±0.008
No gastropods	0.029±0.006	0.015±0.004	0.020±0.003	0.024±0.007

all prey, $P < 0.01$). These differences in vertical prey distribution may in part account for the observed movement of bluegills and other fish species from the slope to the bench region of Lawrence Lake in late May (Hall and Werner 1977)

As in the vegetation, the abunance of large prey in the sediments declined throughout the summer (Table 2). Prey ≥3.0 mm body length declined significantly from 32 358/m³ in May to 15 050/m³ in June, 15 802/m³ in July, and 11287/m³ in August (ANOVA, $P < 0.05$). The total biomass of prey available, however, showed no significant change across the season (Table 7) (ANOVA: all prey, $P > 0.10$; nongastropod prey, $P > 0.10$).

TABLE. 7. Mean number (and dry weight) per metre cubed of major prey groups found in the sediments (5 samples per date). Total prey numbers, total prey biomass, and mean prey weights are ±1 SE.

	Sediment prey no./m^3 (mg dry wt/m^3)			
	May 21	June 14	July 19	Aug. 23
Cladocera	2262 (7)	1500 (30)	10537 (118)	10500 (157)
Ostracoda	9787 (264)	750 (21)	17312 (419)	2262 (59)
Hyalella	1500 (228)	0	2262 (28)	0
Hydracarina	3012 (222)	3012 (230)	3012 (197)	7525 (473)
Chironomidae	34612 (1138)	69225 (1290)	142225 (2368)	156525 (1073)
Other Diptera	56437 (312)	27087 (241)	84275 (235)	35362 (2518)
Tricoptera	0	0	0	750 (5)
Ephemeroptera	5262 (3705)	750 (79)	0	9787 (1620)
Odonata	1500 (1483)	0	0	0
Total Insecta	100837 (6779)	97075 (1610)	232525 (2603)	202425 (5059)
Gastropoda	4512 (4451)	2262 (1841)	0	750 (148)
Total no.	132437±19450	108363±15800	285950±24100	229512±34337
Total biomass	(12025±4325)	(3737±1250)	(3475±437)	(6075±2712)
Mean prey wt (mg)	0.091±0.030	0.034±0.011	0.012±0.001	0.0261±0.011

Discussion

This study represents a first attempt to quantify the total size distribution of invertebrate prey found in a natural lake. Results demonstrate that within each major habitat type the distribution of prey sizes closely matches the lognormal curve, large prey being rarer than small prey. The work of Werner (1977) represents the only other study which has attempted to determine the size distribution of prey in a freshwater system (a series of small experimental ponds), and Werner also found the distribution of prey sizes (weights) to be lognormal. Schoener and Janzen (1968) and Hemmingsen (1934), both studying terrestrial insect communities, similarly report insect body lengths to be lognormally distributed. Thus, there is growing evidence that the body size distributions of insects and other invertebrates in both aquatic and terrestrial environments correspond to the lognormal curve. Although it is expected that prey size distributions will differ quantitatively between lakes, the general lognormal shape of the distribution and the seasonal progression towards smaller prey sizes found in the littoral habitats of Lawrence Lake should prove to be common features of many temperate lakes.

This ability to characterize prey availability by a simple, two-parameter distribution should prove extremely useful in many theoretical examinations of fish foraging, in particular those related to fish bioenergetics and the potential for competition between species or size-classes on the food size dimension of the niche. Werner (1977), for example, measured the time required for bluegills, green sunfish (*L. cyanellus*), and largemouth bass (*Micropterus salmoides*) to pursue and handle prey of different sizes. He then utilized the total distribution of prey sizes available to the fish, along with the estimates of pursuit and handling times, to calculate diet overlaps and potential competitive interactions between the species. Recently, I have used a foraging model based upon the energetic gain and loss of bluegills feeding on different sizes of prey to predict successfully the prey size selection and habitat use of bluegills in the field (Mittelbach 1981). These studies and others (Goss-Custard 1977) demonstrate how a knowledge of the available prey size distribution can be used to generate testable predictions of a species' habitat use and prey selection in the field.

The seasonal reduction in the abundance of large prey in both the vegetation and sediment habitats and the seasonal decline in the total biomass of vegetation prey (exclusive of gastropods) are striking features of the Lawrence Lake data. Other workers have reported a reduction in the total volume or biomass of littoral prey from May to August (Ball and Hayne 1952; Anderson and Hooper 1956; Gerking 1962; Phoenix 1976) but few studies have related these changes to the foraging behavior and prey consumption of fish. Figure 1 shows that the amount of prey ingested by bluegill sunfish feeding in the vegetation of Lawrence Lake over the summer paralleled the amount of invertebrate prey available. As prey biomass in the vegetation declined so did the stomach contents of fish feeding predominantly from that habitat. The bluegill dominates the fish community of Lawrence Lake (68% of total fish biomass (Werner et al. 1977)) and littoral invertebrates constitute a major fraction of its diet (Gerking 1962; Keast 1978; G. G. Mittelbach, W. K. Kellogg Biological Station and Department of Zoology, Michigan State University, Hickory Corners, MI 49060, USA, unpublished data).

Prey abundance and foraging intake are clearly correlated (Fig. 1); however, other factors besides a decline in prey abundance may be responsible for the decrease in the amount of prey ingested by the bluegills. For example, the foraging intensity of bluegills may simply decline over the summer after the energetic demands of spawning and replacing winter weight loss have been met. However, a seasonal reduction in feeding intensity does not appear to be the case in Lawrence Lake; bluegills which shifted from feeding in the vegetation to feeding on zooplankton in early July maintained stomach fullnesses approximately equal to those obtained in the vegetation in May (Mittelbach 1981). Also, in experimental pond studies where resource levels remained high from July to September, bluegills showed little reduction in feeding rate (E. E. Werner et al., W. K. Kellogg Biological Station and

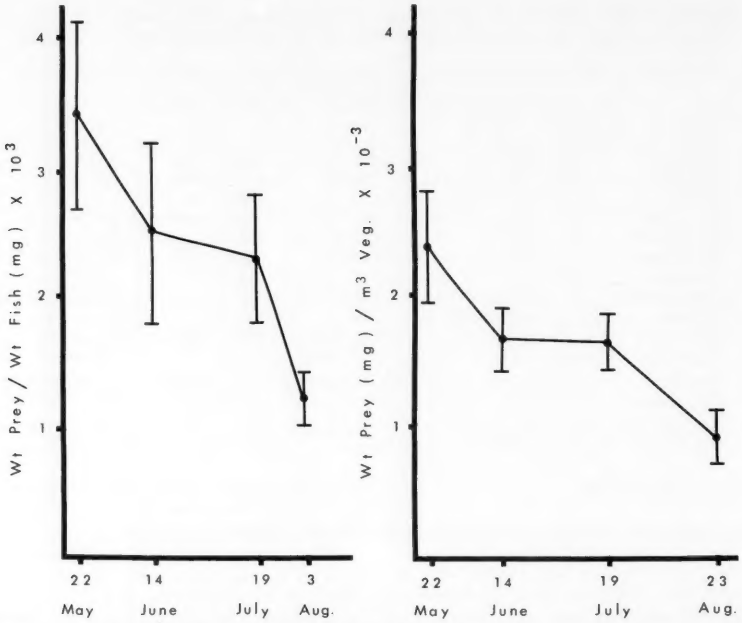

FIG. 1. Comparison of the dry weight of vegetation-dwelling prey in the stomachs of bluegills over the season and the total dry weight of prey available in the vegetation habitat (excluding gastropods). Data points are means ± 1 SE. Bluegills ranged in size from 16 to 143 mm SL, were collected 3/4–1 1/2 hr post-sunrise, and represent those individuals whose stomachs contained >80% vegetation-dwelling prey by weight. Sample sizes were 15–18 fish per date, 10 prey samples per date. For complete details of fish collection and stomach analysis, see Mittelbach (1981).

Department of Zoology, Michigan State University, Hickory Corners, MI 49060, USA. unpublished data). Therefore, the seasonal decline in foraging intake from the vegetation habitat would appear to be due to a reduction in prey availability and not simply a decline in feeding intensity.

Total prey biomass does not completely descibe the profitability of a resource to the consuming species, as numerous studies have shown that the way in which total prey biomass is distributed among prey sizes is very important in determining a fish's foraging intake and growth (Paloheimo and Dickie 1966; Kerr 1971; Wankowski and Thorpe 1979). In relation to this one should note that the decline in bluegill stomach contents from May to August is correlated with a decline in the abundance of large (≥3.0 mm) vegetation-dwelling prey as well as with the decline in total prey available. The decline in the abundance of these large prey may be very important as Hall et al. (1970) have demonstrated a significant correlation between the standing crop of invertebrates >0.01 mg dry weight and the standing crop of bluegills in a series of small experimental ponds. Notably, these workers found no correlation between total production of benthos and the production of fish. Studies of fish foraging and production therefore need to incorporate both size and abundance in characterizing available prey resources.

The seasonal decline in vegetation prey biomass and the shift to smaller prey sizes in Lawrence Lake may be due to size-selective predation by fish (Stráskraba 1965; Hall et al. 1970; Zaret 1980; Mittelbach 1981) or the emergence of late instars and the appearance of new generations, or both. In any case these changes in prey size distribution are correlated with a seasonal reduction in stomach contents (Fig. 1) and potential net energetic return (Mittelbach 1981) for bluegills feeding in the vegetation. Other workers have reported similar declines in fish stomach contents from May through August in temperate lakes (Seaburg and Moyle 1964; Keast 1970; Wilsmann 1979; Laughlin and Werner 1980), and bluegill growth rates also generally decrease across the summer (Gerking 1966). The potential link between seasonally declining resource abundance (biomass and size) and declining foraging intakes and growth of fish is clear and strongly suggestive of resource limitation in these systems. However, further work is needed to establish a causal basis to these relationships and evaluate the impact of asynchronous changes in resource abundance between habitats.

Patterns in prey abundance found in this study are most directly comparable to the results of Wohlschlag (1950), who examined a small marl lake in Indiana (Table 8). His estimates of total prey biomass are considerably less than those reported here for both the vegetation and bare marl habitats. Estimates of total prey biomass determined by Phoenix (1976) are also

TABLE. 8. Total biomass (mg dry wt/m^2) of invertebrate prey found in the littoral habitats of small lakes by various researchers.

	Total prey biomass (mg dry wt/m^2)					
	Phoenix (1976)	Wohlschlag (1950)[a]		Gerking (1962)[b]	This study	
	Vegetation (*Eleocharis*)	Vegetation (*Scirpus*)	Bare marl	Vegetation (*Chara*)	Vegetation (*Scirpus*)	Bare marl
May		359	29		1319	962
June	281	329	30		1473	299
July	85	257	181	1320	1428	278
Aug.	42	332	156	817	801	486

[a]Biomass determined as wet weight; converted to dry weight by multiplying by 0.10. Gastropods not included in samples.

[b]Average biomass for 2 yr (1955, 1956) sampled from 0 to 2-m depth stratum.

far below those measured in Lawrence Lake. However, Phoenix calculated prey biomass using "average weights for representative sizes of organisms" and suggested for this reason that his estimates of biomass are "useful only for showing general trends." The work of Gerking (1962) represents probably the best attempt at determining the biomass of available prey in terms of employing an efficient sampling device and a large number of samples. Clearly from Table 8 there is good agreement between Gerking's estimates of prey biomass (measured in a northern Indiana lake) and those determined in this study. However, a caveat is in order regarding these comparisons. Each of the above studies differed in the method used to sample invertebrates, and the lakes also differed in vegetation type; therefore, differences in invertebrate abundance between lakes may be due to a number of factors. Despite these difficulties, it is still of importance to evaluate the effectiveness of this study relative to others in sampling the total abundance of invertebrates. Prey biomass measured in the vegetation and bare sediments of Lawrence Lake was equal to or greater than estimates of prey biomass previously determined for other small temperate lakes (Table 8) and this indicates that the general patterns of habitat-specific prey abundances and size distributions shown are sound.

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Culture à dialyse : emploi de fibres creuses dialysantes pour la culture massive de phytoplancton[1]

PIERRE MARSOT, RÉAL FOURNIER, ET CLAUDE BLAIS

INRS-Océanologie, 310 des Ursulines, Rimouski, (Qué.) G5L 3A1

MARSOT, P., R. FOURNIER ET C. BLAIS. 1981. Culture à dialyse : emploi de fibres creuses dialysantes pour la culture massive de phytoplancton. Can. J. Fish. Aquat. Sci. 38: 905–911.

Un nouveau système de culture à dialyse pour organismes photosynthétiques a été développé. Son fonctionnement est basé sur l'emploi d'unités dialysantes autonomes, fixées à la chambre de croissance. Il s'agit de cartouches de fibres creuses dialysantes (hémodialyseurs) remplaçables et dotées d'une très grande surface d'échange osmotique. Ce dispositif permet de réaliser, pour la première fois, des cultures volumineuses d'algues en dialyse, sur une période prolongée, avec un rendement comparable à celui obtenu dans des petits sacs à dialyse. De plus, cette méthode assure une production d'algues nettement supérieure aux autres procédés conventionnels que nous avons testés (cultures statique et semi-continue).

Mots clés: culture à dialyse, phytoplancton

MARSOT, P., R. FOURNIER ET C. BLAIS. 1981. Culture à dialyse : emploi de fibres creuses dialysantes pour la culture massive de phytoplancton. Can. J. Fish. Aquat. Sci. 38: 905–911.

The apparatus we developed for the dialysis culture of phytoplankton utilizes disposable hollow dialysis fibers (hemodialyzer) as a separate dialysis unit coupled to a growth chamber. This system provides a large-scale dialysis culture of longer duration and similar biomass values when compared to microalgae grown in small dialysis tubing. The yield of algae obtained with our dialysis system represent an important improvement over the conventional batch cultures tested.

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AU cours des dernières décennies, une panoplie de méthodes de culture d'algues unicellulaires, statique et continue [*batch and continuous cultures*] a été développée tant en recherche appliquée (Burlew 1953; Johnston 1976; Goldman et Ryther 1975; Grobbelaar 1979; Tamiya 1973; Brunel et al. 1950; Palacio 1977; Guillard 1975; De la Cruz et Alfonso 1975; Loosanoff 1951; Palmer et al. 1975) que pour des études fondamentales sur la photosynthèse (Retovsky 1966; Jüettner 1977; Carpenter 1968). Le fonctionnement des méthodes décrites par ces auteurs, repose à la base, sur l'utilisation par les algues des nutrilites du milieu jusqu'à leur point de tarissement, et par le renouvellement automatique (méthode dite, continue) ou manuel (semi-continue) de la masse biologique produite par du milieu neuf. On peut concevoir que le rendement de ce système devienne rapidement limité par la capacité nutritionnelle du substrat nourricier.

Le procédé de culture en dialyse conçu originalement pour les bactéries, croissance in situ ou in vitro (Metchnikoff et al. 1896; Carnot et Fournier 1900) et appliqué plus tard aux organismes photosynthétiques (Pratt 1944; Pallotta et al. 1955; Trainor 1965; Jensen et al. 1972; Prakash et al. 1973; Dor 1975a, b) s'avère un moyen beaucoup plus efficace, particulièrement lorsque le volume du milieu ambiant est infini (Berland et al. 1976). En fait, il est considéré à la fois, comme un système ouvert et stérile grâce à l'enveloppe dialysante composant les parois de l'enceinte. Dans de telles conditions, les cellules ne sont plus restreintes à leur propre milieu, mais peuvent également « extraire » leurs nutriments du liquide environnant via les processus de l'osmose. Cette diffusion procure aux cellules un apport continu des éléments nutritifs et une élimination de tout instant de leurs métabolites autoinhibiteurs (Jensen et al. 1972; Prakash et al. 1973). Également, l'accroissement des échanges osmotiques accompagnant l'évolution d'une culture à dialyse favorise la prolongation de la phase exponentielle et permet d'atteindre une densité cellulaire beaucoup plus importante (Dor 1975a, b; Schultz et Gerhardt 1969).

Jusqu'à présent, la conception des appareils à dialyse, tant pour les cultures de bactéries que pour celles d'organismes photosynthétiques, était fondée sur l'emploi d'une membrane dialysante servant, totalement ou en partie, de paroi d'enceinte et de surface d'échanges osmotiques. Ce procédé introduit dans le fonctionnement de ce type de culture des limitations techniques majeures concernant la production massive d'algues sur une longue période. En effet, il parait alors impossible d'atteindre de fortes densités cellulaires dans une culture de grand volume, en raison de la baisse inévitable du

[1]Brevet déposé.

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rapport superficie d'échange sur le volume de culture (Jensen 1976). La disponibilité de surface d'échange pour chaque cellule devient plus restreinte provoquant ainsi une réduction du transport des nutriments et des métabolites inhibiteurs. Par ailleurs, la vulnérabilité des membranes aux bactéries et diatomées cellulolytiques (Vargo et al. 1975) rends impraticable la culture à long terme car les systèmes à dialyse développés à ce jour ne permettent pas le remplacement des membranes sans mettre un terme définitif à la croissance des algues.

La méthode de culture à dialyse que nous décrivons dans ce rapport permet de contourner ces difficultés expérimentales. Le système comporte une chambre de croissance de 10 L, dotée d'unités dialysantes distinctes, en l'occurence des cartouches de fibres creuses renouvelables. L'appareil permet de maintenir une culture aseptique sur une longue période avec un taux de croissance comparable à celui réalisé, par d'autres auteurs, dans les petits sacs à dialyse. Le rendement de cette méthode (croissance cellulaire et production de biomasse) représente une amélioration importante sur les systèmes de cultures conventionnels, non-dialysants.

Matériel et méthodes

L'algue test *Phaeodactylum tricornutum*, qui a servi à ces expériences, nous a été gracieusement offerte par le Dr Subba Rao de l'Institut Océanographique de Bedford. La souche stérile était maintenue en culture statique, sur milieu F/2 de Guillard et Ryther (1962) et utilisée au cours de la phase logarithmique pour ensemencer les enceintes de culture. Le volume d'inoculation variait entre 40 et 60 mL. Les cultures à dialyse ont été menées au laboratoire humide de Pointe-au-Père. Les enceintes étaient orientées vers le sud-ouest et recevaient un éclairage naturel tamisé, d'intensités variables; en moyenne, 18 000 lx pour les journées ensoleillées et 6000 lx durant les ennuagements. Leur milieu de croissance, de l'eau de mer naturelle en circulation constante, était prélevée à 5 m de profondeur et filtrée sur cartouches de filtres Gelman jusqu'à 1.0 μm. Elle contenait de 5 à 12 μg-at./L de N et 0.5 à 2.0 μg-at./L de P, avec des valeurs de salinité variant entre 25 et 29‰. Lors d'une expérience, l'eau de mer a été enrichie (milieu F/2) et maintenue dans un bassin nourricier de 100 L, en circuit fermé.

Mesure des indices de croissance

Les comptages cellulaires ont été effectués sur une lame à puits calibrée et les concentrations en chlorophylle *a* déterminées par fluorimétrie (Strickland et Parson 1972). Les valeurs de poids sec ont été obtenues par une lyophylisation (72 h) des échantillons d'algues recueillis sur filtres prépesés de polycarbonate. Les mesures de concentration en ions nutritifs (NO_3-N et PO_4-P) furent réalisées à l'aide d'un appareil Technicon autoanalyser II.

Appareil de culture

Le montage de notre système de culture à dialyse est schématisé par la figure 1. L'appareil est constitué de deux principaux éléments: la chambre de croissance des algues et les unités dialysantes. L'enceinte de croissance, d'une capacité de 10 L est faite de Plexiglas et possède une forme cylindrique (19.5 cm sur 44 cm de hauteur). Elle est logée dans un plus grand bassin d'eau thermostatée (12 ± 0.5°C), contrôlée par une unité réfrigérante (non indiquée sur la figure). La chambre de croissance est en communication avec une ou plusieurs unités dialysantes grâce à des tubes de transfert (inférieur et supérieur) traversant le bassin thermostaté. Un tuyau d'arrivée d'air ou d'un mélange air/CO_2 est greffé au tube de transfert inférieur. L'enceinte est dotée également, d'une sortie de gaz munie d'une trappe à bactéries (filtre à disque 0.2 μm de Gelman), d'un bouchon d'ensemencement en caoutchouc et de tubulures pour le prélèvement et la vidange.

L'unité dialysante utilisée au cours de ces expériences, était l'hémodialyseur, modèle Disscap, construit par la compagnie Hospal Med. Corp. Littleton, USA. Il s'agit d'une cartouche de plastique transparent contenant des milliers de fibres creuses de collodion (200 μm) pouvant offrir une surface d'échange de 1.1 m^2. Elles permettent une plus grande diffusion des molécules possédant une masse molaire inférieure à 1500. De légères modifications ont été apportées à l'hémodialyseur: nous avons obscurci la cartouche de manière à empêcher toute croissance des algues sur les fibres et le colmatage des espaces interfibrillaires, et réduit l'ouverture d'évacuation de l'eau de mer à 1.5 mm afin d'exercer une pression satisfaisante dans les fibres. La cartouche est remplacée régulièrement (tous les 4 à 8 jours, selon le degré de contamination de l'eau) afin d'éviter la biodégradation des fibres et la contamination de la culture.

Fonctionnement du système de culture

La circulation du milieu de culture dans le système prend naissance au niveau du tube de transfert inférieur. Elle est générée par un jet d'air ou un mélange d'air et de CO_2 (5%) envoyé dans ce tube. Le barbotage du gaz agit tel un siphon [*air lift pump*] et entraîne la culture vers l'unité dialysante où les échanges osmotiques s'effectuent. La culture s'achemine ainsi entre les fibres dialysantes de la cartouche et se retrouve, finalement, à la partie supérieure de la chambre de croissance (fig. 1). De plus, cet apport gazeux a pour effet d'activer la croissance des algues (Stein 1973) et donne une meilleure homogénéité à la culture. À l'aide d'un débit-mètre, ce débit gazeux est ajusté à 0.8 L/min de manière à produire une circulation de 10 L/h et un "turnover" de la culture de 24 cycles·jour^{-1}/dialyseur. Une courbe de calibration a été déterminée à cette fin (fig. 2). L'eau de mer ou le milieu nourricier, circule à l'intérieur de chaque fibre de l'unité de haut en bas, c'est-à-dire dans un courant inverse à la culture, favorisant ainsi les échanges osmotiques. Un débit-mètre et un manomètre installés juste avant la cartouche dialysante régularisent le flux d'eau de mer dans les fibres ainsi que la pression. Ils servent en même temps à contrôler le niveau dans l'enceinte. Le maintien d'un niveau fixe est réalisé en exerçant une pression intrafibrillaire égale à la pression extrafibrillaire, cette dernière correspondant au poids de la colonne de culture dans la chambre de croissance. À cette fin, une pression de 60 mm Hg suffit à maintenir le système en équilibre. Par ailleurs, lors du renouvellement d'un échantillon par du milieu neuf, la pression dans les fibres doit être supérieure

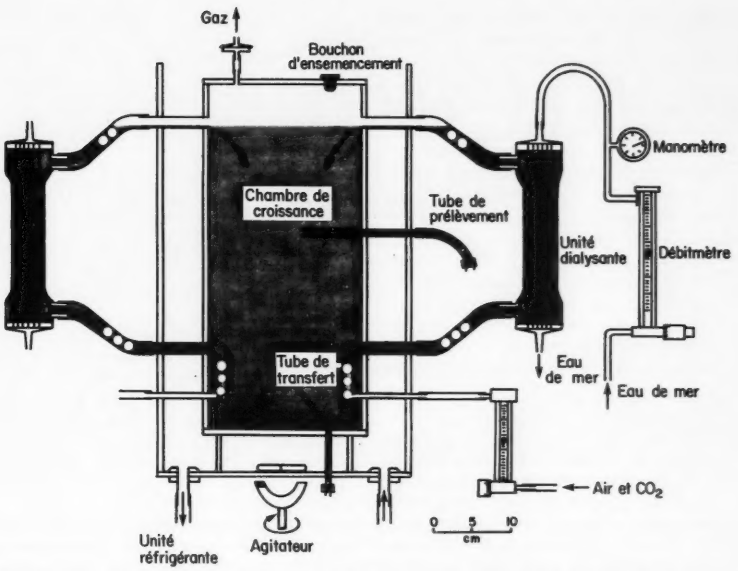

FIG. 1. Montage d'un appareil de culture à dialyse basé sur l'emploi d'unités isolées de fibres creuses dialysantes (hémodialyseur).

à celle de l'enceinte. Dans ce cas, on ajustera la pression à 150 mm Hg dans les deux unités dialysantes. Le remplissage du cylindre s'effectuera alors à la vitesse de 15 à 20 cm/jour.

Résultats

CROISSANCE DE *P. TRICORNUTUM* DANS UN SYSTÈME À FIBRES DIALYSANTES : COMPARAISON AVEC LES PROCÉDÉS CONVENTIONNELS

Sur une période de 12 jours nous avons mesuré la croissance de *P. tricornutum* en terme de nombre cellulaire, de la concentration en chlorophylle *a* et du poids sec dans trois systèmes de culture maintenus dans des conditions expérimentales identiques. Le premier dispositif comprenait une enceinte munie de deux hémodialyseurs, le deuxième d'un seul dialyseur, enfin le troisième, imitant le système de culture conventionnel (méthode semi-continue), fonctionnait en milieu clos sans unité dialysante. Dans ce cas, le renouvellement du volume d'échantillonnage par de l'eau de mer stérile était réalisé par filtration (minicapsule Gelman 0.2 μm). On observe à la figure 3, des différences importantes entre les cultures à dialyse et la culture conventionnelle, différences encore plus prononcées lorsque les deux dialyseurs sont en opération. En fait, nous notons des augmentations de l'ordre de 23, 19 et 12 fois pour les comptages cellulaires, la concentration en chlorophylle *a* et le poids sec, respectivement. Soulignons aussi, l'accroissement significatif de la production, près du double dans l'ensemble des paramètres, lorsque la culture est exposée à une surface d'échange deux fois plus grande. Que l'on compare ici la production obtenue

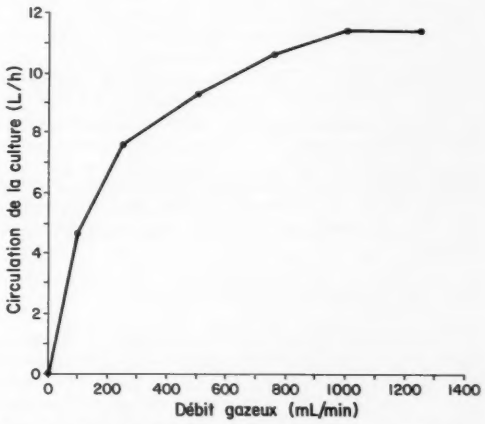

FIG. 2. Circulation (L/heure) de la culture dans l'unité dialysante en fonction du débit gazeux (air ou air/CO_2).

avec les dispositifs munis d'une et de deux unités dialysantes.

Selon les fluctuations saisonnières d'éclairage et de concentration en nutrilites dans l'eau de mer, notre appareil à dialyse permet d'atteindre des densités cellulaires s'échelonnant entre 5 et 15×10^6 cellules/mL, avec des débits d'eau de mer dans le dialyseur variant entre 5 et 6 L/h. L'ajout de sels nutritifs à l'eau de mer (mélange F/2 de Guillard et Ryther 1962) et de gaz carbonique (5%) dans l'air de circulation assure une population de 37×10^6 cellules/mL et un poids sec

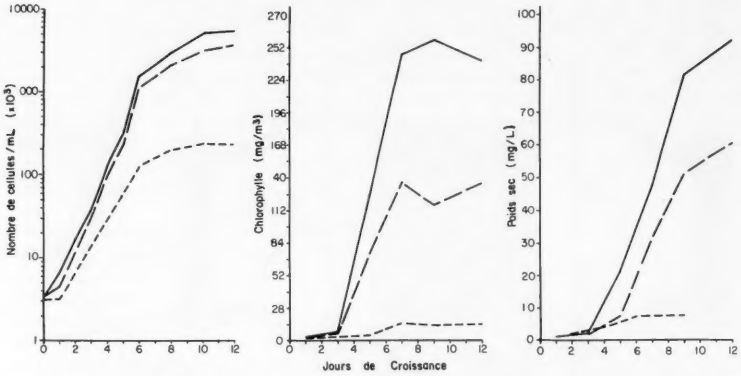

FIG. 3. Croissance de *Phaeodactylum tricornutum* dans un système de culture à dialyse doté de deux unités dialysantes (—) et d'une seule unité (– –) et dans un système conventionnel (culture semi-continue) (---). Les indices de croissance choisis sont le comptage cellulaire, la concentration en chlorophylle *a* et le poids sec. Les cultures ont été menées simultanément, et aseptiquement, en utilisant une eau de mer non-enrichie. Le débit de l'eau dans le dialyseur était ajusté à 5 L/h.

TABLEAU. 1. Diffusion des sels nutritifs (NO_3-N, PO_4-P) dans un système de culture à fibres dialysantes durant une période de 24 h. Les échantillons d'eau de mer, à l'entrée et à la sortie de l'hémodialyseur, ainsi que du milieu de culture, ont été prélevés simultanément. Au moment de l'échantillonnage, la culture était en phase linéaire atteignant 4×10^6 cellules/mL. Le débit de l'eau de mer dans les fibres était ajusté à 6.5 L/h.

	Eau de mer (entrée du dialyseur)		Eau de mer (sortie du dialyseur)		Diffusion vers la culture		Concn. dans la culture (mg/L)
	Concn. (mg/L)	Poids total (mg/jour)	Concn. (mg/L)	Poids total (mg/jour)	Total (mg/jour)	Pourcentage	
N	0,26±0,10	40,56	0,12±0,04	18,72	21,84	54	0,011±0,005
P	0,048±0,01	7,49	0,037±0,01	5,87	1,62	22	0,0175±0,009

correspondant à 1 g/L. En culture statique avec le même médium enrichi, la croissance de cette algue a plafonné à 3.5×10^6 cellules/mL en moyenne, avec un poids sec de 130 mg/L.

ÉCHANGES OSMOTIQUES DES ÉLÉMENTS NUTRITIFS

Une estimation de la diffusion des nitrates et des phosphates à travers les fibres dialysantes a pu être établie durant la phase linéaire d'une culture de *P. tricornutum*. Des échantillons d'eau de mer et du milieu ont été prélevés simultanément durant 24 h, à l'arrivée et à la sortie du dialyseur ainsi que dans la chambre de croissance. Le tableau 1 présente les valeurs des échanges osmotiques dans ce système à dialyse, exprimées en concentration par millilitre et en poids absolu par jour. Ainsi, pour les nitrates et les phosphates, le transport ou le taux d'utilisation par la culture représente, respectivement, 54 et 22 % du débit journalier maintenu dans l'unité dialysante. L'écart-type important que nous avons obtenu s'explique par les fluctuations obsrvées pour les concentrations en sels nutritifs de l'eau de mer naturelle. Mentionnons toutefois que cette diffusion, mesurée à partir des 24 prélèvements, a toujours été positive, c'est-à-dire orientée vers la culture. Le tableau 1 indique aussi et de façon remarquable, l'important écart de concentration ionique dans l'eau de mer à l'arrivé (colonne 1) et dans la chambre de croissance (colonne 4) soit, un facteur de 24 pour NO_3 et 2.8 pour PO_4.

De la même façon, des variations de pH ont été suivies durant 24 h, dans le milieu de culture et dans l'eau de mer, avant et après la dialyse (fig. 4). Durant la période photoactive particulièrement, les valeurs de pH à la sortie de l'échangeur sont constamment plus élevées, d'environ 0.5 unité, que celles de l'eau de mer à l'entrée du dialyseur. Cette activité des fibres semble vouloir se refléter au niveau de la culture opérant avec le dialyseur, par le maintien d'un pH plus physiologique durant la période d'activité photosynthétique. Remarquons que cette même culture privée de son dialyseur subira, durant les heures suivantes, une hausse appréciable de son pH (fig. 4).

Discussion

La méthode de culture à dialyse présente de l'avis de plusieurs phycologues, des atouts intéressants sur les autres procédés conventionnels, car elle ouvre de nouvelles possibilités de recherche notamment dans les domaines de l'écologie et de

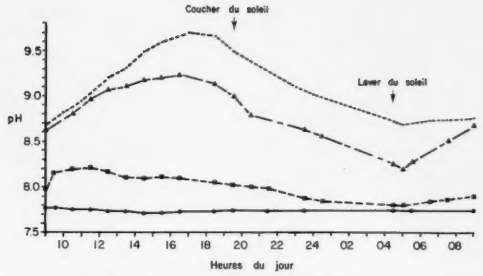

FIG. 4. Variations du pH d'une culture de *Phaeodactylum tricornutum* opérant avec une unité à dialyse (—▲—); la même culture privée du dialyseur, le jour suivant (--△--). Mesures du pH de l'eau de mer, à l'arrivée (—●—) et à la sortie du dialyseur (--■--). Le débit de l'eau de mer dans le dialyseur a été ajusté à 4.7 L/h. Durant les 48 h, la culture a connu des conditions d'éclairage semblables (deux jours ensoleillés) et a reçu de l'air sans CO_2.

la productivité biologique. Ainsi, l'utilisation de cultures d'algues tests, incubées in situ dans des enceintes dialysantes (sac à dialyse, ou autres), constitue une approche attrayante dans la détermination des facteurs nutritionnels contrôlant la production primaire des eaux (Jensen et al. 1972; Berland et al. 1976; Sakshaug et Jensen 1978; Kossut et Maestrini 1977). Le système à dialyse est également bien adapté pour des études sur les effets subléthaux de certains métaux traces dans le milieu (Jensen et Rystad 1974), et sur la caractérisation de substances ectocrines libérées par les organismes photosynthétiques (Pratt 1944). Dans le secteur de la production de protéines algales à partir d'eaux usées, cette méthode s'avère très prometteuse en raison de son rendement supérieur (Dor 1975a, b; Palotta et al. 1955) et des avantages techniques qu'offre ce procédé: la membrane dialysante sert de protection efficace contre les microorganismes indésirables (exemple, les bactéries entériques) et maintient la culture dans un état aseptique tout en permettant la diffusion des éléments stimulants.

Si la culture à dialyse est utilisée depuis fort longtemps (Metchnikoff et al. 1896) elle n'a pourtant pas connu de développements techniques valables, notamment en ce qui concerne la production massive d'algues [*large-scale culture*]. Toujours, elle fut destinée à des cultures de très petits volumes: sacs à dialyse de 18 mm de diamètre (Jensen et al. 1972; Berland et al. 1976) ou des enceintes ne contenant guère plus de 150 à 350 mL de milieu (Jensen et al. 1972; Jensen 1976; Dor 1975). En fait, le handicap majeur de la méthode réside principalement dans la baisse du rapport surface d'échanges/volume de culture, rencontrée dans les cultures de plus grand volume. C'est une difficulté qui est liée à la conception même du procédé voulant que la membrane dialysante, tenant lieu d'échanges osmotiques, serve également de parois à l'enceinte. À ceci s'ajoute, évidemment, le sérieux inconvénient de la biodégradation de la membrane de collodion par les bactéries cellulolytiques (durée de vie, 10−14 jours; Vargo et al. 1975), et de l'impossibilité de la remplacer sans détruire la culture. Dans le but de résoudre ces difficultés de base, Jensen (1976) recommande une séparation physique des deux fonctions vitales de la culture à dialyse, i.e. la croissance et les échanges osmotiques: « *The first improvement seems to be a separation of the photosynthetic from the dialysis step in the system. This will open up the way for more materials to be used in the dialysis membrane.* »

La méthode que nous décrivons dans ce rapport préconise justement cette distinction des deux activités métaboliques du phytoplancton: d'une part, l'étape « échanges de nutrilites » réalisée par l'unité dialysante (cartouche de fibres creuses) et d'autre part, l'étape « croissance-photosynthèse » s'accomplissant dans une enceinte de capacité variable. Les résultats que nous avons obtenus avec *P. tricornutum* (ex: densité cellulaire de 37×10^6 cellules/mL, 1 g/L de poids sec), montrent sans aucun doute qu'un tel dispositif à dialyse peut fournir une productivité élevée sur une grande échelle. Cette production est comparable à celle de 40×10^6 cellules/mL, obtenue dans des petits sacs à dialyse avec le même organisme et un milieu de croissance semblable (Prakash et al. 1973). Une croissance analogue voire même supérieure, de cette diatomée, a été produite dans une enceinte plus volumineuse encore (15 L), par l'ajout d'un troisième dialyseur (résultats non publiés). À la lumière de ces résultats, il est permi de croire que notre méthode de culture pourrait maintenir ce taux de croissance dans des volumes quasiment illimités, pourvu qu'on y ajoute la surface d'échange requise afin de garder un rapport S/V élevé. Cette hypothèse semble être corroborée par les résultats de la figure 3 indiquant une production accrue d'algues avec l'ajout d'unités dialysantes.

Notre méthode de culture présente une autre amélioration technique appréciable sur l'ancien système à dialyse; c'est celle de réaliser le remplacement des surfaces d'échanges dialysantes de façon aseptique et sans altérer l'évolution d'une culture. C'est un avantage de la méthode qui nous a permi de maintenir durant plusieurs semaines et tout au long des expériences des cultures aseptiques d'algues, et ce, malgré l'emploi d'une eau de mer contaminée. Cette manipulation est facile car l'appareil possède des unités dialysantes indépendantes, donc remplaçables, ce qui n'est évidemment pas le cas des autres méthodes où la membrane à dialyse constitue la paroi de l'enceinte.

Grâce aux nouvelles facilités d'échantillonnage de la méthode, nous avons estimé, pour la première fois, l'intensité des échanges osmotiques des principaux éléments nutritifs du phytoplancton dans un système à dialyse. On remarque d'une façon générale une utilisation presqu'instantanée et exhaustive des sels nutritifs par le moyen des fibres creuses. Celle-ci est particulièrement évidente pour les nitrates où une disparition supérieure à 50% est mesurée (tableau 1) durant le séjour relativement court de l'eau de mer dans les fibres (35 s pour un débit ajusté à 6.5 L/h). L'énorme différence de concentration entre la culture et le milieu nourricier (facteur de 24 pour les nitrates) met également en évidence cette utilisation efficace des cellules, et peut-être aussi, explique-t-elle la diffusion rapide des sels à travers le faisceau de fibres. Une diffusion aussi intense semble vouloir se produire pour les ions HCO_3 dont une estimation indirecte a été faite par les mesures de pH. Ces échanges se traduisent par un niveau de pH plus bas de 0.5 à 0.7 unité pour la culture munie d'un dialyseur durant la période photoactive. Puisque le pH de l'eau de mer est largement déterminé par la concentration d'ions bicarbonates (Parsons et al. 1977), les résultats obtenus ici signifient un apport important du HCO_3-/CO_2 de l'eau de

mer vers la culture. Ce transport d'ions carbonates favorise ainsi les processus biologiques directement reliés à la fixation même du carbone inorganique c'est-à-dire la photosynthèse (Frank et Loomis 1949).

À l'appui de ces résultats nous pouvons conclure que la culture massive de microorganismes photosynthétiques est réalisable par le moyen de la dialyse, pourvu que les deux étapes vitales, diffusion nutritive et photosynthèse, constituent deux entités bien distinctes. Il devient alors possible de produire une biomasse très dense de phytoplancton, sur une grande échelle, simplement par l'ajout d'unités dialysantes. D'autres algues unicellulaires, notamment *Dunaliella tertiolecta* (Chlorophyceae) et *Pavlova* (*Monochrysis*) *lutheri* (Chrysophyceae), ont été cultivées avec succès par ce procédé.

Les perspectives d'application de cette méthode sont nombreuses. Pour des études biochimiques et écophysiologiques du phytoplancton, la méthode assure un prélèvement facile des échantillons (algues, eau de mer à l'entrée du dialyseur, eau de mer et les métabolites cellulaires à la sortie). Elle trouvera également une application rapide en aquiculture et dans le secteur de la production de protéines algales à partir d'eaux usées domestiques. Dans ce dernier contexte, un dispositif à fibres non-biodègradables pourrait maintenir sans risque de contamination une culture d'algues sur une période très longue, en dépit d'un milieu nourricier contaminé.

Remerciements

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Effect of the Algal Length/Aperture Length Ratio on Coulter Analyses of Lake Seston[1]

H. A. VANDERPLOEG

Great Lakes Environmental Research Laboratory, NOAA, Ann Arbor, MI 48104, USA

VANDERPLOEG, H. A. 1981. Effect of the algal length/aperture length ratio on Coulter analyses of lake seston. Can. J. Fish. Aquat. Sci. 38: 912–916.

Experiments on sizing seston and nylon rods in Coulter apertures of different sizes showed that a major difficulty in obtaining a continuous particle-size spectrum for lake seston is the presence of long, thin algae which are significantly undersized unless their lengths are shorter than aperture length. These fibers affect not only the choice of the largest aperture tube required but also the combination of aperture tubes necessary to obtain a continuous particle-size spectrum. Methods are given for selecting aperture tubes and blending the data obtained from them to give a best estimate of the continuous particle-size spectrum.

Key words: Coulter counter, multitube technique, lake seston, particle-size analysis, aperture tube selection

VANDERPLOEG, H. A. 1981. Effect of the algal length/aperture length ratio on Coulter analyses of lake seston. Can. J. Fish. Aquat. Sci. 38: 912–916.

Quand on mesure la taille du seston et celle de bâtonnets de nylon dans les ouvertures de diverses longueurs d'un compteur de Coulter, on constate qu'il est très difficile d'obtenir une gamme continue de tailles de particules de seston lacustre en présence d'algues longues et minces, dont la taille est toujours sous-estimée, à moins qu'elles ne soient plus courtes que la longueur de l'ouverture. Ces fibres influencent non seulement le choix du tube à ouvertures maximales requis, mais aussi la combinaison de ces derniers nécessaire à l'obtention d'un spectre continu de tailles de particules. Nous décrivons des méthodes permettant de choisir les tubes et d'assembler les données de façon à obtenir la meilleure estimation d'un gamme continue de tailles de particles.

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COULTER counters have been used extensively in both oceanography and limnology for determining seston-size distributions (e.g. Sheldon and Parsons 1967a, b; Carder et al. 1971; Sheldon et al. 1972; Haffner and Evans 1974) and zooplankton grazing (e.g. Parsons et al. 1967b; Poulet 1973, 1974; Richman et al. 1977; Vanderploeg 1981), but have limitations (e.g. Karuhn et al. 1975; Thom et al. 1969; Kachel 1976) in accurate sizing. I found that a major difficulty in sizing lake seston is the presence of long, thin algae which are significantly undersized unless their lengths are shorter than aperture length. These fibers affect not only the choice of the largest aperture tube required but also the combination of aperture tubes necessary to obtain a continuous particle-size spectrum.

To measure seston-size distributions in Lake Michigan water for a study of zooplankton grazing (Vanderploeg 1981), I used a 16-channel model TA II Coulter counter connected via a specially constructed three-way switch to three sample stands, each with a different-sized aperture tube. Using this device, I quickly obtained multitube analyses required for obtaining a continuous-size spectrum of seston (Sheldon and Parsons 1967a, b) having equivalent spherical diameters (ESDs) between 1 and 160 μm. In these analyses, a single aperture tube can sense particles whose ESDs are between 2 and 40% of aperture diameter (Coulter Electronics 1975). Two or more aperture tubes must be used to extend the size range of analysis to cover the particle-size range of 1 to 160 μm ESD. To blend the results from two tubes, there must be overlap in channels, or size categories, between aperture tubes. Size categories shared by each of the two tubes are collectively called the overlap region.

To evaluate the multitube technique for Lake Michigan seston, I used methods similar to those used by Haffner and Evans (1974). They reasoned that two aperture tubes of different sizes give correct results that are useful for blending if each aperture tube yields the same counts per millilitre in each of the channels of the overlap region. For brevity of expression, close agreement between apertures will be described hereafter as good count overlap and poor agreement as poor count overlap. Since Haffner and Evans obtained good count overlap for River Thames water with 100- and 200-μm aperture tubes, they concluded that these tubes were correctly sizing the seston there. In contrast to their results, I obtained poor count overlap in many channels for analyses of 53-μm-screened Lake Michigan water with various combinations of 50-, 140-, 200-, and 400-μm-diameter aperture tubes. (Screening was necessary to prevent plugging of the 50-μm aperture.)

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In this paper, I document the count overlap problem and show that its probable cause is the presence of long fiber-shaped algae in Lake Michigan that are undersized in the shorter sensing zones of the smaller aperture tubes. To demonstrate this point quantitatively and determine the proper ratio of algal length to aperture length for correctly sizing the algae, I determined the volumes of unimodal distributions of long, thin, nylon rods with apertures of different sizes. These electronically determined volumes were then compared with absolute volumes determined from length and diameter measurements obtained with a microscope. From the experiments with the seston and with the rods, I make recommendations on choice of aperture tubes for analyzing seston-size distributions that contain fiber-shaped algae.

Methods

Most analyses of lake or river water were made on samples freshly collected in fall 1976 and summer and fall 1977. Lake Michigan water samples were collected from a depth of 4 m at a station 15 km west of Grand Haven, MI. The river water came from a depth of 1 m in the Grand River 2 km from Lake Michigan. Some analyses were made with water that had been preserved with 1% Formalin. Particle-size spectra obtained for fresh samples and related Formalin-preserved subsamples of these samples were very similar.

Lake and river water were screened through a 53-μm Nitex screen that was glued to the bottom of a Plexiglas tube. To assure gentle, efficient screening, the tube sat within a slightly larger-diameter container that was allowed to fill with water. Screened water was drained from the larger container through a hose near the bottom. This device was used to assure gentle, efficient screening of lake water, with minimum damage to algae passing through the screen. In the context of the present study, the smooth flow of water through the screen, allowing alignment of fibers in the direction of the flow, may have allowed more efficient passage of long, thin algae through the screen than would have been obtained by pouring water through a conventional screen.

Samples were analyzed with each aperture tube according to general methods outlined by Sheldon and Parsons (1967a, b) and Coulter Electronics (1975). Before analysis with a particular aperture tube, 25% (w/w) NaCl solution was pipetted into a sample of water to make a final salt concentration of 0.5% (Mulligan and Kingsbury 1968; Evans and McGill 1970; Haffner and Evans 1974). Background counts on filtered lake water samples were negligible.

To facilitate multitube analyses, a special switch box was constructed that allowed switching between three sample stands, each with a standard Coulter aperture tube of different diameter. The control switch is a 12-position non-shorting ceramic rotary switch (Central Lab No. CRL PA-2013) that is axially coupled to a 12-position hermetically sealed signal switch (Leeds and Northrup No. 031263) of low resistance, inductance, and capacitance. The first three positions of this pair of switches are connected to the sample stands, and the wipers are connected to the Coulter counter.

Nylon rods of 200- and 500-μm length were made by microtoming bundles of nylon yarn having individual strands 13 μm in diameter on a Mico 202-B fiber microtome. In experiments with 200-μm rods, the concentration was adjusted for coincidence values of about 3% (as given by the CI meter) when analyzed with a 400-μm aperture tube. Triplicate subsamples were analyzed with the 400-, 200-, and 50-μm aperture tubes, and a portion of the suspension was preserved with 1% Formalin for later microscopic examination. The same procedure outlined for the 200-μm rods was used with the 500-μm rods; however, measurements with the 50-μm aperture were not possible because of plugging by the rods. Actual concentrations and volumes of the rods were determined from examination with an inverted microscope. To determine the frequency distribution of rod volumes, I determined the length and diameter of about 200 rods, using a standard ocular micrometer to measure the lengths and a Unitron filar micrometer eyepiece to measure diameters. Measurement precision obtained with these micrometers was better than ±2%.

Results and Discussion

Figure 1 summarizes results of all overlap experiments performed with lake water samples and other materials with 50- and 200-μm-diameter aperture tubes. The ratios of counts per millilitre observed by the 200-μm aperture tube to counts per millilitre observed by the 50-μm aperture tube in the various size categories of the overlap region are shown. Perfect count overlap is indicated by a ratio of 1. In general, this ratio increases with increasing size of particles. In contrast, much better count overlap was obtained for diluted water from the Grand River, for a suspension of dried sediment from the bottom of the Grand River, and for various concentrations of suspensions of SR-WR 1-40 μm microspheres (available from Particle Information Service, Grant's Pass, OR). In all these experiments shown in Fig. 1, coincidence was less than 3% (as indicated by CI meter on the instrument).

Other experiments showed that the count overlap problem was not caused by coincidence or dead time (count saturation) effects arising from the shape of the particle-size spectrum (Bader et al. 1972). First, several experiments showed that count overlap and concentration (corrected for dilution) were unchanged by dilution. Second, excellent count overlap (with 50-, 140-, and 400-μm apertures) was obtained for mixtures of plastic microspheres whose particle-size spectra were constructed to mimic closely those lake water spectra giving particularly bad overlap. These results and the results of Fig. 1 imply that the poor count overlap is caused by the unique nature of the particles in lake water. Thus, the problem is reduced to explaining how a given particle will give rise to different sensed volumes in different-sized aperture tubes.

Microscopic examination of 53-μm-screened lake water used in overlap experiments showed that algae having the form of long, thin fibers were more abundant in samples yielding poor overlap. Dominant elongated genera present included *Oscillatoria*, *Anabaena*, *Synedra*, *Melosira*, *Rhizosolenia*, and *Fragilaria*. It is noteworthy that algae having lengths of hundreds of micrometers passed through the 53-μm screen. Because of the broad range of particle shapes and sizes in these samples, no attempt was made to determine the particle-size spectrum microscopically for comparison with electronic results. Leslie (1978) details the potentially

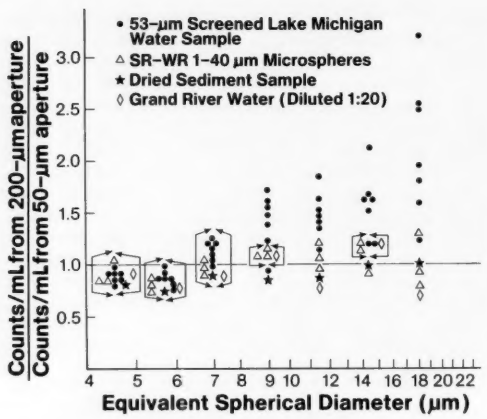
• 53-μm Screened Lake Michigan Water Sample
△ SR-WR 1-40 μm Microspheres
★ Dried Sediment Sample
◊ Grand River Water (Diluted 1:20)
Counts/mL from 200-μm aperture / Counts/mL from 50-μm aperture
3.0
2.5
2.0
1.5
1.0
0.5
4 5 6 7 8 9 10 12 14 16 18 20 22
Equivalent Spherical Diameter (μm)

FIG. 1. Count overlap obtained for Lake Michigan seston and other materials using 200- and 50-μm-diameter apertures.

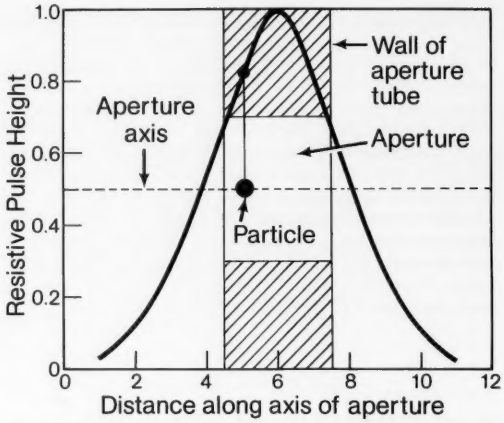

FIG. 2. Resistive pulse (in relative units) generated by a small sphere moving along the axis (in relative units) of an aperture. Figure was schematized from figures of Eckhoff (1969) and Kachel (1976).

large errors associated with such microscopic measurements. The nature of particles in lake water and a review of the papers on principles of electronic sizing of particles (e.g. Gregg and Steidley 1965; Eckhoff 1969; Grover et al. 1969; Kubitschek 1969; Thom et al. 1969; Zimmerman 1974a, b; Davies et al. 1975; Karuhn et al. 1975; Kachel 1976) suggested two possible explanations for poor count overlap. The first hypothesis argues that elongated algae in lake water samples were undersized by the shorter sensing zones of the smaller aperture tubes. The second hypothesis argues that dielectric breakdown of cell membranes of naked flagellated algae (Zimmermann et al. 1974a, b) in the more intense electric fields found in smaller apertures led to undersizing these algae in smaller apertures. This breakdown and subsequent partial conduction of current through the cells give rise to undersized pulses. Because breakdown is caused by the potential to which a cell is exposed, larger cells break down at lower field strengths, because they span a larger region of the field. Thus, dielectric breakdown is consistent with the overlap pattern observed. However, this phenomenon was not expected to be significant since field strengths in all apertures used was less than 5×10^3 V·cm^{-1}, the threshold level at which this phenomenon usually becomes significant (Zimmermann et al. 1974a, b). Moreover, experiments at different field strengths did not change the overlap pattern for lake seston. Comparisons of count overlap were made at 1×, 2×, and ½× usual levels by changing applied aperture currents. Thus, the first hypothesis appears to be the only acceptable explanation of the poor count overlap.

Since the larger aperture tubes give more correct results for elongated particles, the question arises as to how long must an aperture be relative to algal length to size it properly. The standard apertures I used were cylindrical holes having a length/diameter ratio of about 0.75. Electric field strength measured along the axis of the aperture is highest at the center and decreases symetrically in each direction along the axis to values near zero at some distance before the aperture entrance and after the aperture exit (e.g. Gregg and Steidley 1965; Eckhoff 1969; Thom et al. 1969; Kachel 1976). This field shape results in the round-topped resistive pulse (Fig. 2) that is observed for a particle as it passes through the aperture (e.g. Eckhoff 1969; Thom et al. 1969; Kachel 1976). As a result of the shape of this field, a sphere or a long rod coaxially aligned with the aperture will yield the greatest signal in the center of the aperture as it moves along the axis (Fig. 2). When a long rod is at the center, much of its volume will be in regions of much less than maximal field strength, leading to a pulse that is undersized. Since neither the precise nature of the electric field nor the paths the particles took was known, it was impossible to specify a priori the reduction in signal strength resulting from various rod length/aperture length ratios. Thus, the ratio to size the rods correctly was determined by comparing electrical volumes of the monosized nylon rods in apertures of different sizes with volumes determined from microscopic measurements. Algae were not used in these experiments because their noncircular cross sections make volume estimates from microscopic measurements of their dimensions subject to considerable error.

Tables 1 and 2 compare electronically determined size frequencies of 200- and 500-μm rods with microscopically determined size frequencies. In both cases, there was excellent agreement between electronic and microscopic estimates of number of rods per millilitre. Size categories are given as ESDs and corresponding length. Corresponding length is the length of a mean diameter (12.61 μm) rod having the given ESD. Percent frequency in a size category is given in terms of both number and total volume of rods. Percent frequency by volume was determined directly from the Coulter counter percent volume output. As would be expected for the 200-μm rods (Table 1), microscopic measurements indicated most rods were in the 137- to 274-μm-length range. The 7.6% by number and 16% by volume of rods in the 274- to 549-μm-length range are the result of pairs of rods stuck together along their length.

Tables 1 and 2 clearly demonstrate that rods will be signifi-

TABLE 1. Size frequency of 200-μm-long nylon rods as measured microscopically and by the Coulter counter using three different aperture tubes. Percentage values without parentheses are percentages by number; values in parentheses are percentages by volume.

Size category, equivalent diameter (μm)	Corresponding length range (μm)	Percentage in a size category							
				Coulter counter aperture diameter (μm)					
		Microscope		50		200		400	
20.2–25.4	34.4–68.7	0.0	(0.0)	100	(100)	1.9	(1.1)	1.9	(1.0)
25.4–32.0	68.7–137	3.5	(1.9)	—	(—)	65.7	(58.2)	29.5	(21.6)
32.0–40.3	137–274	88.9	(82.1)	—	(—)	30.7	(36.6)	64.6	(68.2)
40.3–50.8	274–549	7.6	(16.0)	—	(—)	1.6	(4.1)	4.0	(9.2)

TABLE 2. Size frequency of 500-μm-long nylon rods as measured microscopically and by the Coulter counter using two different aperture tubes. Percentage values without parentheses are percentages by number; values in parentheses are percentages by volume.

Size category, equivalent diameter (μm)	Corresponding length range (μm)	Percentage in a size category					
				Coulter counter aperture diameter (μm)			
		Microscope		200		400	
25.4–32.0	68.7–137	0.0	(0.0)	11.2	(8.1)	**1.9**	(1.0)
32.0–40.3	137–274	1.4	(0.7)	84.1	(81.0)	**29.6**	(23.2)
40.3–50.8	274–549	89.4	(84.0)	4.3	(9.2)	**62.6**	(62.4)
50.8–64.0	549–1098	9.2	(15.3)	0.4	(1.8)	**5.9**	(13.4)

cantly undersized if their lengths are longer than the aperture length. Both 400- and 200-μm aperture tubes severely undersized the 500-μm rods (Table 2). In particular, the frequency distribution of 500-μm rods with the 200-μm aperture was shifted more than one channel downward relative to the microscopic results. The 200-μm rods were severely undersized by both 50- and 200-μm aperture tubes (Table 1). In the case of the 200-μm aperture, the frequency distribution of rods was shifted almost one channel downward relative to the microscopic results. Since the 20.2-μm size category is the last channel for the 50-μm aperture, it really corresponds to the >20.2-μm size category for the 50-μm aperture; thus, it sensed the 200-μm rods as being larger than 20.2 μm. The 200-μm rods were only slightly undersized by the 400-μm aperture. These results suggest that long, thin algae will be only slightly undersized by the Counter counter if the aperture length is greater than particle length. These results corroborate results of Sheldon and Parsons (1967b), who observed that the microscopically estimated volumes of two marine chain-forming diatom species having lengths up to 300 μm compared well with their electronic volumes given by a 400-μm aperture tube. Since an aperture properly sizes spheres whose diameters are less than 40% of aperture diameter (Coulter Electronics 1975), it follows that for very careful sizing work, rod length should be less than 40% of aperture diameter. In this regard, experiments of Andrews and Irvine (1969) suggested that the mean volume of 20-μm-diameter by 100-μm-long nylon rods was accurately determined with a standard 280-μm-diameter aperture tube.

The results of all these experiments with elongated particles run counter to the expectation that elongated particles will be undersized because their shape factors are less than those of the microspheres used to calibrate the apertures (e.g. Thom et al. 1969; Kachel 1976). These shape factors predict that the elongated rods used in my study and the 20 × 100 μm rods will give rise to respective electrical volumes (1/1.5) times and (1.2/1.5) times true volume (e.g. Gregg and Steidley 1965; Grover et al. 1969; Thom et al. 1969; Kachel 1976). The shape factor effect may have been mitigated by edge effects and particle rotation or orientation in the aperture (e.g. Thom et al. 1969; Davies et al. 1975; Karuhn et al. 1975; Kachel 1976).

To blend data from multitube analyses, I used the following technique, which is similar in principle to the methods recommended by the instrument manufacturers (Coulter Electronics 1975; E. V. Hoff, Coulter Electronics, Inc., Hialeah, FL, personal communication; R. F. Karuhn, Particle Data Ltd., Elmhurst, IL, personal communication). This method makes use of the observations that the large (longer) aperture tubes more correctly size the longer particles and that count overlap for lake water is invariably best in the smallest size categories of the overlap region (e.g. Fig. 1). Calculate count per millilitre data for all aperture tubes used. Start with the largest size category of the largest tube, move down the size categories and accept all data from the largest aperture tube until two adjacent size categories are reached that have the best collective count overlap (e.g. Fig. 1). From these two channels, the channel of best overlap is selected as the crossover channel. The concentration in the crossover channel is taken as the mean of the values for the larger and smaller tube. For all smaller-size categories, all data are taken from the smaller aperture tube unless another crossover point is reached, if a third, still smaller tube was used. If so, the same procedure described for the larger tubes is used for the two smaller tubes. An alternative method of blending would be to accept all count per millilitre data from the larger tube and use

only those results from the smaller tube that extend the size range of analysis to size categories smaller than those that can be sensed by the larger tube.

The choice of aperture tubes used is dictated by the nature of the seston in lake water. First, the largest tube selected must be chosen so that aperture length is greater than length of the longest common algae. Second, the size difference between apertures used is affected by the shape of the seston. Experiments have shown that 50- and 400-μm aperture tubes cannot be used to analyse the particle-size spectrum of lake water between 1 and 160 μm because there are no size categories of good count overlap. A 200- or 140-μm aperture must be added to obtain good count overlap in at least one channel. In contrast, excellent count overlap was obtained with 50- and 400-μm aperture tubes for mixtures of microspheres mimicing the lake water spectra. Experience has shown that when analyzing lake water, data usually cannot be blended from two tubes if the ratio of the diameter of the larger aperture tube to the smaller is greater than 4.

For the purpose of comparing results of different aperture tubes, the water in the experiments reported here was screened through a 53-μm screen in the apparatus described. When the total size spectrum for particles between 1 and 160 μm was required for 153-μm-screened lake water used in the zooplankton grazing experiments, the water was screened through a 153-μm screen in the apparatus described above before analysis with the Coulter counter. Further screening was only required before analysis on the 50-μm aperture tube to prevent aperture plugging. This screening was accomplished by pouring the water through an ordinary 53-μm Nitex screen.

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Uptake of Cadmium in Perfused Rainbow Trout (*Salmo gairdneri*) Gills

P. PÄRT AND O. SVANBERG[1]

University of Uppsala, Institute of Zoophysiology, P.O. Box 560, S-751 22 Uppsala, Sweden

PÄRT, P., AND O. SVANBERG. 1981. Uptake of cadmium in perfused rainbow trout (*Salmo gairdneri*) gills. Can. J. Fish. Aquat. Sci. 38: 917–924.

Uptake of cadmium in gills from rainbow trout (*Salmo gairdneri*) has been studied by the head perfusion technique. The importance of checking the viability of the perfused gills is considered and some viability criteria are discussed. The results show that cadmium is transferred through perfused gills. A 10-fold increase in the external Cd concentration from 5×10^{-8} to 5×10^{-7} mol·L^{-1} resulted in a nearly 100-fold increase in cadmium transfer. At higher cadmium concentrations the uptake rate of the element diminished after having reached a maximum within 30 min. A complexing agent, EDNTA, which strongly chelates free cadmium ions decreases the transfer of cadmium through the gills. The advantage of using in vitro techniques instead of conventional uptake experiments is discussed and it is concluded that the gill perfusion technique is a useful tool in studies of factors affecting heavy metal uptake in fish.

Key words: perfusion technique, fish gills, *Salmo gairdneri*, cadmium uptake, viability, EDNTA

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La perfusion céphalique a été la technique utilisée pour étudier l'absorption du cadmium dans les branchies de la truite arc-en-ciel (*Salmo gairdneri*). En plus de souligner l'importance de vérifier la viabilité des branchies perfusées, les auteurs examinent certains critères de viabilité. Les résultats démontrent qu'il y a transfert de cadmium à travers les branchies perfusées. Une augmentation de 10 fois de la concentration du Cd extérieur, soit de $5 \cdot 10^{-8}$ à $5 \cdot 10^{-7}$ mol · L^{-1}, entraîne une augmentation de près de 100 fois de transfert de cadmium. À des concentrations de cadmium plus élevées, le taux d'absorption diminue après avoir atteint un sommet en dedans de 30 min. Un agent complexant, l'AEDNT, qui lie fortement les ions de cadmium libre, ralentit le transfert de cadmium à travers les branchies. Les auteurs discutent des avantages de la méthode in vitro par rapport aux expériences conventionnelles d'absorption. Ils concluent que la perfusion branchiale est utile dans l'étude des facteurs qui influent sur l'absorption des métaux lourds par les poissons.

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LITTLE is understood about the mechanisms behind the uptake of heavy metal ions from water in fish and other branchiate organisms. It is, however, well documented that water quality, such as hardness (e.g. Howarth and Sprague 1968; McCarthy et al. 1978), salinity (e.g. Eisler 1971; O'Hara 1973), and soluble organic substances (e.g. Giesy et al. 1977), affect the toxicity of solved metals to these organisms. A question not fully understood is to what extent the effects are due either to altered availability, depending on changes in the chemical state of the metal, or to biological factors affecting the sensitivity of the organisms.

A common indirect method in studies of heavy metal uptake is analyzing the metal content in the organisms after exposure. As the concentration in the organisms is the net sum of uptake and elimination, difficulties arise in the interpretation of the results. Owing to large individual variations, this indirect method also demands a large number of analyses to obtain a statistically valid sample. Consequently, when the investigator is interested only in uptake mechanisms, it is more convenient to work with the uptake site isolated.

In teleosts, the skin, gut, and gills are in direct contact with the external water and can therefore be considered potential uptake sites of solved substances. However, as the skin is nearly impermeable to both water and solved substances and as at least freshwater fishes do not drink water (Maetz 1974), an uptake of solved substances via these routes seems to be of minor importance. On the other hand, the gills of teleosts are specialized for gas and ion exchange with the aqueous envi-

[1]Present address: The National Environmental Protection Board, Brackish Water Toxicology Laboratory, Studsvik, S-611 82 Nyköping, Sweden.

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ronment (Randall 1970; Maetz 1974). They have a large, effectively irrigated area, which together with a short distance between blood and water facilitates these transfers (Hughes 1972). This implies that the gills also serve as the main uptake site for other dissolved compounds including heavy metals.

The purpose of this study was to find a suitable method to investigate transfer of cadmium in fish gills under controlled conditions. Using this method we will try to evaluate the mechanisms behind cadmium transfer in the gills and especially focus on how the quality of the water outside the gills influences the penetration of the metal.

Perfusion techniques enable the study of the function of an isolated organ under controlled conditions (Ross 1972). Perfused gill preparations have been used to study gill Na^+ and Ca^{2+} regulation (Payan et al. 1975; Girard and Payan 1977a, b; Milhaud et al. 1978; Millet et al. 1979), water and solute transfer (Haywood et al. 1977; Isaia et al. 1978), and gas exchange (Wood et al. 1978; Haswell et al. 1978), but corresponding information about heavy metal uptake in perfused gills is rare. We believe that this preparation will be useful when trying to understand in more detail the mechanisms behind heavy metal uptake by fish gills. In the present investigation, we have adopted the perfused head preparation of Payan and Marry (1975) in studies in cadmium uptake in rainbow trout (*Salmo gairdneri*) gills. The uptake rate has been measured during the time course of the perfusion, in different Cd^{2+} concentrations in the water and in the presence of a chelating agent. The importance of checking the viability of the perfused gills is considered and some different criteria of viability are discussed.

Materials and Methods

Fish

Rainbow trout, 150–220 g, were used in all experiments. They were obtained from a local dealer, kept at the institute in 1000-L tanks with running aerated Uppsala tap water (Table 1), and fed with commercial trout food (Trout feed extra, Astra-Ewos, Sweden). The temperature in the holding tanks ranged between 10 and 12°C.

Gill Preparation

The isolated head of rainbow trout was prepared according to the technique described by Payan and Matty (1975). Briefly, the fish was decapitated behind the operculum and the ventral and dorsal aortas were cannulated with polyethylene catheters (Intramedic PE 90 and PE 190, Clay-Adams Inc., USA). The eight gill arches were simultaneously perfused by connecting the ventral aortic catheter to the perfusion pump delivering the perfusion medium. The medium leaving the gills was collected from the catheter in the dorsal aorta. During perfusion, the head was placed in a cylindrical box permitting circulation of water over the gills without leakage.

The surgical procedures performed prior to perfusion were carried out with the gills effectively irrigated by aerated tap water. Average time for each of 34 preparations was 11.5 min, measured from the time the fish was caught in the aquarium until the beginning of the perfusion. The gills were not irrigated for less than 1 min during this time.

TABLE. 1. The composition of Uppsala tap water during the experimental period July 26 to October 24, 1979.

Temperature	10–12°C
pH	7.6
HCO_3^-	4.3 mmol·L^{-1}
Cl^-	0.6 mmol·L^{-1}
F^-	0.1 mmol·L^{-1}
SO_4^{2-}	0.6 mmol·L^{-1}
NO_3^-	0.1 mmol·L^{-1}
Na^+	0.6 mmol·L^{-1}
K^+	0.1 mmol·L^{-1}
Ca^{2+}	2.8 mmol·L^{-1}
Mg^{2+}	0.4 mmol·L^{-1}

Perfusion Setup

Initially, the head was perfused with a Ringer solution at a constant flow, ranging between 1.8 and 2.6 mL · min^{-1} · (100 g fish)$^{-1}$ with the aid of a peristaltic pump (Minipuls HP 4, Gilson, France). Later, when experience was gained, a cardiac pump, built at the institute with a Harvard model 1405 cardiac pump as a prototype, was used, delivering 0.90–1.3 mL · min^{-1} · (100 g fish)$^{-1}$ at 50 beats · min^{-1}. These flows correspond to the wide range of cardiac output values measured in vivo for rainbow trout (Wood et al. 1978). The pressure in the ventral aorta was measured by a pressure transducer (P23Db, Gould Statham Instruments Inc., USA) connected to a potentiometric recorder (Servograph, Radiometer, Denmark).

Perfusion Media

The perfusion medium used was designed for freshwater eels by Rankin and Maetz (1971). It is suitable for rainbow trout as well (P. Payan, Group Biologic Marine Villefranche/Mer France, personal communication). It contained 1 g · L^{-1} glucose, 40 g · L^{-1} PVP-40 (polyvinylpyrollidone, MW = 40 000, Sigma Inc., USA) as a plasma protein substitute and 5000 IE · L^{-1} sodium heparin (Vitrum, Sweden) as an anticoagulant. When not otherwise stated, the medium contained 1×10^{-5} mol · L^{-1} atropine (atropine sulphate, ACO, Sweden) and 1×10^{-6} mol · L^{-1} epinephrine (adrenaline bitartrate, ACO, Sweden).

According to Haswell et al. (1978), the Ringer was not gassed. The pH remained stable at 7.6 after adjustment with carbon dioxide. Before use, the buffer was filtered through a 1.2-μm filter (Millipore Inc., USA).

External Media

The external medium consisted of 200 mL oxygenated tap water (Table 1). It was pumped through the buccal cavity at a rate of 1.5 L · min^{-1} to maintain an effective flow over the gills. It was bubbled with air throughout the experiment.

A total of 3.7×10^5 Bq ^{109}Cd ($^{109}CdCl_2$, 1.85×10^6 Bq · μg^{-1}, Radiochemical Center, Amersham) was added together with varying amounts of carrier Cd^{2+} ($Cd(NO_3)_2$, Merck, BRD) to the external medium. In some experiments, the medium also contained 2.9×10^{-5} mol·L^{-1} EDNTA (Na_2-(ethylenedinitrilo)tetraacetate, Titriplex III, Merck, BRD).

The sodium concentration in the medium was continuously measured by a Na^+-sensitive electrode (Electric Instruments Ltd., U.K.) and a millivolt-meter (PHM 26, Radiometer, Denmark).

Calibration curves were made by standard additions of Na^+ to tap water. Both the external and the perfusion media were maintained at 10°C by a thermostat unit (Heto, Denmark).

Experimental Procedure

The head was perfused for 10−15 min to obtain a blood-free preparation. The typical experiment was started with the addition of ^{109}Cd to the external medium. It lasted for either 60 or 90 min divided in 15-min periods. When not otherwise stated, the perfusion medium was collected from the dorsal aorta during the 15-min periods and the flow was determined gravimetrically.

In the beginning of the experiment and at the end of each 15-min period, 0.5-mL samples of the external medium were counted in a scintillation counter (model 1185, Nuclear Chicago, USA) and the potential of the sodium electrode was recorded.

Calculation of Cd Influx

The ^{109}Cd in the perfusion medium collected from the dorsal aorta during a 15-min period was concentrated in a 2-mL ion-exchange resin (Chelex-100, Bio Rd Lab., USA). The resin completely trapped all ^{109}Cd in 25−30 mL medium. It was counted in the scintillation counter for 10 000 counts.

The Cd influx was calculated from:

$$F_{Cd} = \frac{^{109}Cd\ i \cdot Q \cdot 60}{^{109}Cd_e^*}$$

F_{Cd} = influx of Cd in $mol \cdot h^{-1} \cdot (100\ g\ fish)^{-1}$, $^{109}Cd\ i$ = ^{109}Cd in perfusion medium in dorsal aorta in $cpm \cdot mL^{-1}$, Q = perfusion flow in $mL \cdot min^{-1}$ $(100\ g\ fish)^{-1}$, and $^{109}Cd_e^*$ = specific activity of ^{109}Cd in external medium in $cpm \cdot mol^{-1}$.

Sodium balance and cadmium uptake values are presented as means ± standard deviation of mean. The Wilcoxon rank sum test was used for statistical comparison.

Histology

Pieces of the first right gill arch were cut from perfused heads, fixed in 2.5% glutaraldehyde in 0.1 $mol \cdot L^{-1}$ Na-phosphate buffer (pH = 7.4), followed by 1% osmium tetroxide in 0.1 $mol \cdot L^{-1}$ phosphate buffer (pH = 7.4), and, after dehydration in ethanol, embedded in Epon 812. One-micrometre sections were cut with an ultratome and stained in toluidine blue. Gills from fish in the storage aquaria served as controls.

Viability

Results obtained from perfusion experiments are relevant only if the perfused organ is viable. The criteria of viability used were sodium balance across the gill membrane, histology, and vasomotor activity.

Results and Discussion

Sodium Balance

A freshwater teleost actively resorbs Na^+ by the gills from the external water compensating for a diffusional loss of the ion. The result is a somewhat positive net flux (Maetz 1974). A positive Na^+ net flux in the gills during perfusion, indicating a viable preparation, results in decreasing sodium concentration in the external water whereas an increase indicates either a leaky preparation or a loss of the active Na^+ resorption, or both.

It was found that perfusion with the aid of the peristaltic pump gave big losses of Na^+ ($-490 \pm 309\ \mu mol \cdot h^{-1}$ $(100\ g\ fish)^{-1}$ ($n = 7$)), indicating a nonviable preparation. A change to the pulsatile "cardiac pump" resulted in a much lower loss ($-19 \pm 21\ \mu mol \cdot h^{-1} \cdot (100\ g\ fish)^{-1}$ ($n = 10$)). This Na^+ loss was still too high to be acceptable. Trout heads for these experiments were prepared without irrigation of the gills. When the technique was modified to include flushing of the gills during the preperfusion operations, a net influx of sodium ($+2.0 \pm 8.8\ \mu mol \cdot h^{-1} \cdot (100\ g\ fish)^{-1}$ ($n = 28$)) during a 90-min perfusion was obtained.

This value is comparable to the net fluxes measured for freshwater fish in vivo (Payan et al. 1975).

Histology

Histological examination of the gills before and after perfusion offered another way of checking the viability. Figure 1 compares nonperfused gills (Fig. 1a) with those perfused by the peristaltic pump (Fig. 1b) and by the cardiac pump (Fig. 1c). Perfusion by the former led to remarkable structural changes in the secondary lamellae. Large pieces of epithelium had sloughed off from the underlying basal lamina. It explains the large Na^+ losses in these perfusions. The positive Na^+ fluxes in experiments with the cardiac pump and after preparing the head with the gills irrigated indicate a viable preparation, which is supported by the histological observations. Only minor structural changes can be detected in these gills (Fig. 1c).

Vasomotor Activity

Recordings of the pressure from the ventral aorta give information about the condition of the capillary bed. An impaired capillary blood flow results in an elevation of the perfusion pressure. Without vasoactive substances in the perfusion medium, the pressure in the ventral aorta was around 35−40 mm Hg (1 mm Hg = 133.322 Pa) when the peristaltic pump was used whereas it was 25−30 mm Hg with the cardiac pump. Addition of 1×10^{-6} $mol \cdot L^{-1}$ adrenaline resulted in a characteristic pressure response, an initial pressure rise followed by a drop, which also has been observed by others (Wood 1974; Girard and Payan 1977). It could still be reproduced after 6−8 h of perfusion. A rise of pressure during perfusion was observed when using the peristaltic pump whereas the pressure remained stable in perfusions performed with the aid of the cardiac pump. Compared with the other viability tests discussed, the pressure and response to vasoactive substances such as adrenaline seem to be less sensitive

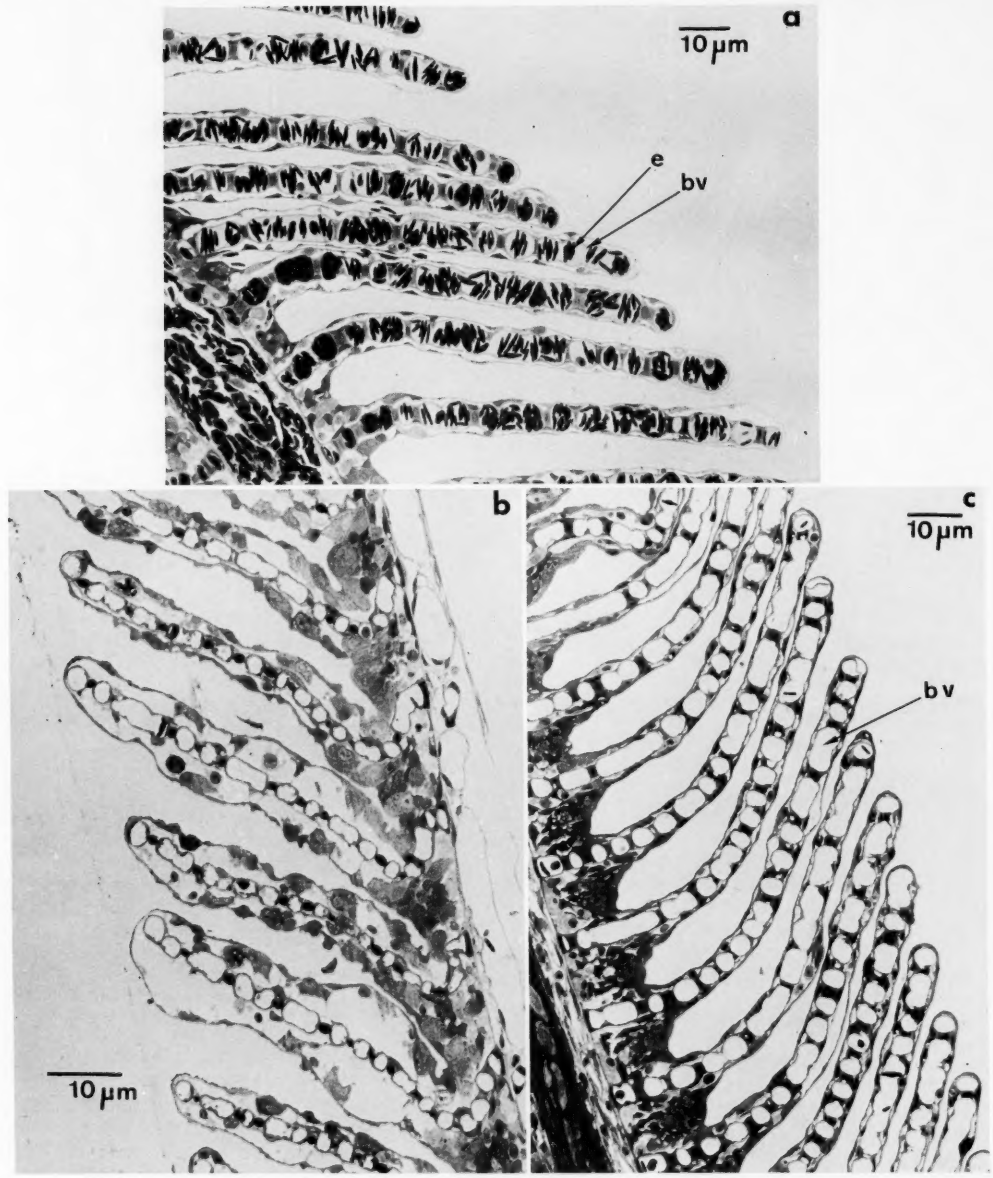

FIG. 1. Histological examination of secondary lamellae from rainbow trout gills before and after perfusion; 1-µm sections stained with toluidine blue. (a) Not perfused gill. Bc = blood vessel with erythrocytes (e). (b) Gill perfused during 30 min with the aid of a peristaltic pump : perfusion flow, 2 mL·min^{-1}·(100 g fish)$^{-1}$; mean perfusion pressure, 38 mm Hg. (c) Gill perfused during 30 min with the aid of a pulsatile pump (''cardiac pump'') : perfusion flow, 1 mL·min^{-1}·(100 g fish)$^{-1}$; mean perfusion pressure, 25 mm Hg. Bc = blood vessel.

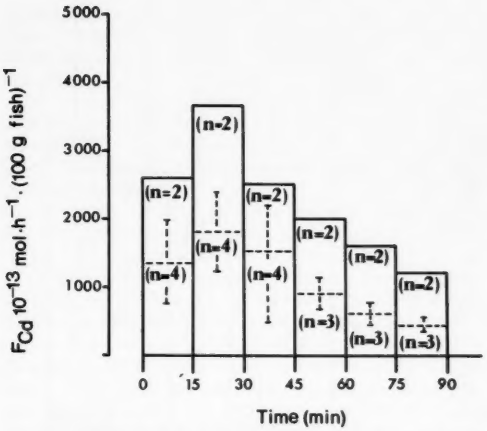

FIG. 2. Influx of cadmium, F_{Cd} (mean ± SD), in mol·h^{-1}·(100 g fish)$^{-1}$ through perfused gills of rainbow trout. The concentration of the metal in the water outside the gills is 5×10^{-6} mol·L^{-1} (560 μg·L^{-1}). Solid lines represent experiments with a positive sodium balance; dotted lines represent experiments with a negative sodium balance. Statistical comparison on paired data between periods 15–30 min and 75–90 min is based on pooled observations from both groups ($P < 0.05$).

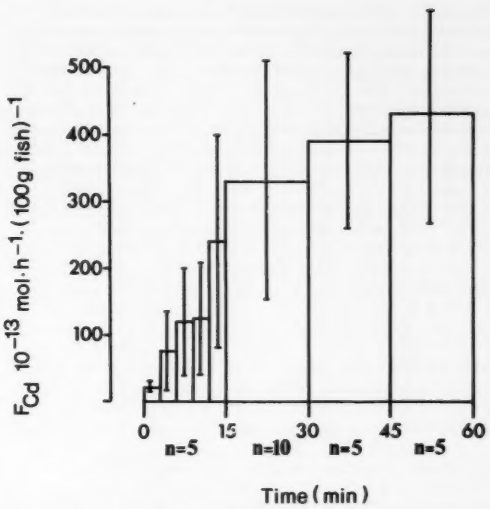

FIG. 3. Influx of cadmium, F_{Cd} (mean ± SD), in mol·h^{-1}·(100 g fish)$^{-1}$ through perfused gills of rainbow trout. The concentration of the metal in the water is 5×10^{-7} mol·L^{-1} (56 μg·L^{-1}). Statistical comparison on paired data between start and pooled observations from period 30–60 min ($P < 0.05$).

as tests for gill condition. This is further emphasized by Ross (1972), who, considering liver perfusion, points out the restricted value of pressure and flow measurements as viability tests, especially when working with the biochemical functions of the tissue.

UPTAKE OF CADMIUM

When discussing cadmium experiments, only perfusions with viable gills, as defined above, are considered. Experiments in which the criteria for viability were not fulfilled generally resulted in lower cadmium uptake values. This tendency is illustrated in Fig. 2. However, further investigations are needed before any conclusions about the relation between cadmium uptake and the energy state of the gills can be drawn.

At external cadmium concentrations ranging from 5×10^{-9} to 5 x 10^{-7} mol·L^{-1}, the influx of cadmium in mol·h^{-1}·(100 g fish)$^{-1}$ rapidly increased during the first 30 min whereupon it tended to stabilize at a steady level (Fig. 3). When the cadmium concentration was increased to 5×10^{-6} mol·L^{-1}, the transfer of cadmium decreased during the course of the perfusion after a maximal value in the second 15-min period (Fig. 2). This trend was more pronounced at an external Cd concentration of 1×10^{-5} mol·L^{-1}. These latter cadmium concentrations are in the range of values at which acute lethal effects are observed in several exposed aquatic organisms (Hammons and Huff 1975).

In fish exposed to acute toxic concentrations of metals such as Cu, Zn, and Cd, structural damage and hyperplasia occur in the gills (Baker 1969; Gardner and Yevich 1970; Skidmore 1970). An impaired gill oxygen transfer is supposed to be the primary cause of death in these fish. Exposure of rainbow trout to Cd (1.3×10^{-6} mol·L^{-1}) and Pb (0.7×10^{-6} mol·L^{-1}) also stimulates mucus production (Varanasi and Markey 1978), which was also observed in the perfused gills at the highest cadmium concentrations. The mucus released to the water could possibly bind the free cadmium ions, transferring them to a less available state and in this way decrease the influx. However, this was not the case as, after renewal of the external medium during the perfusion, cadmium uptake continued to decrease. Obviously the gill permeability to the metal was affected in some way. Damage of secondary lamellae, hyperplasia, and thickening of the gill mucus layer increase the diffusion distance between blood and water. Such an increase in distance could well explain the decreased cadmium transfer in the present perfusions. Four or six gill preparations exposed to this metal concentration were not viable according to Na^+ balance. This indicates that the Na^+ regulation in these gills was affected. However, we are still not convinced that this is an effect of the high Cd concentration.

The transfer of Cd through the gills, in relation to external cadmium concentration, is given in Table 2. A 10-fold increase in external Cd concentration from 5×10^{-8} to 5×10^{-7} mol·L^{-1} resulted in a nearly 100-fold increase in Cd transfer. A further 10-fold increase in concentration to 5×10^{-6} mol·L^{-1} increased the transfer 10-fold. This comparison is based on the second 15-min period when Cd transfer in 5×10^{-6} mol·L^{-1} was maximal. Chertok et al. (1979), using everted sacs of rat small intestine, report a similar relation between cadmium transfer and cadmium concen-

TABLE. 2. Influence of cadmium concentration in the water on the influx of cadmium (F_{Cd}) in perfused gills of rainbow trout. Values obtained during a period 15–30 min after starting the perfusion are compared. Statistical comparison (Wilcoxon test): 5×10^{-8} mol·L^{-1} vs. 5×10^{-7} mol·L^{-1}, $P < 0.05$; 5×10^{-7} mol·L^{-1} vs. 5×10^{-6} mol·L^{-1}, $P < 0.05$.

Cadmium concn. (mol·L^{-1})	F_{Cd} 10^{-13} mol·h^{-1}·(100 g fish)$^{-1}$ (mean ± SD)
5×10^{-9} (0.6 μg·L^{-1})	0.65 ($n = 1$)
5×10^{-8} (5.6 μg·L^{-1})	3.55 ± 2.24 ($n = 6$)
5×10^{-7} (56 μg·L^{-1})	332 ± 179 ($n = 10$)
5×10^{-6} (560 μg·L^{-1})	3648 ± 707 ($n = 2$)
1×10^{-5} (1.12 mg·L^{-1})	3400 ($n = 1$)

tration in the bathing medium. When the concentration of Cd in the bathing medium was low, the transfer of the metal was also low, but after the concentration of the metal in the medium had risen above a certain level, the transfer of the metal rapidly increased. They interpreted their results to mean that a fraction of the metal was bound in the tissue while the remainder was free to diffuse into the serosal fluid. At higher Cd concentrations, the binding sites in the tissue were saturated more rapidly resulting in an increase of free cadmium available to diffuse. The large difference in cadmium transfer in the gills between 5×10^{-8} and 5×10^{-7} mol·L^{-1} could be a result of a similar phenomenon. A disturbing factor in this case is the large variation in Cd transfer between single perfusions under equal conditions. This might be an effect of differences in perfused gill area between preparations. This possibility will be examined in further studies.

The toxicity or availability of cadmium to aquatic organisms is related to the free cadmium ion concentration in the water, which in turn is determined by the total cadmium concentration and the degree of complexation to ligands (Poldoski 1979; Sunda et al. 1979). Increasing salinity or water hardness reduces the toxicity of cadmium to fish and crustaceans (Davies 1976; McCarthy et al. 1978; Sunda et al. 1979). In these situations, the free cadmium ion concentration is decreased by complexation to Cl^-, OH^-, HCO_3^-, and CO_2^{2-}. Consequently, chelating agents, such as ethylenediaminetetraacetate (EDTA) and nitrilotriacetate (NTA), by decreasing the free ion concentration, also reduce the toxicity of heavy metals to fish and crustaceans (Sprague 1968; Sunda et al. 1979; Poldoski 1979). These compounds bind the metal in strong complexes, which do not readily penetrate biological membranes. However, this is not a general rule. The liposolubility of the formed complex is also important in relation to its biological availability. Cadmium uptake in *Daphnia magna* was increased by diethyldithiocarbamate (DDC), which binds the metal in a more liposoluble complex than EDTA and NTA (Poldoski 1979).

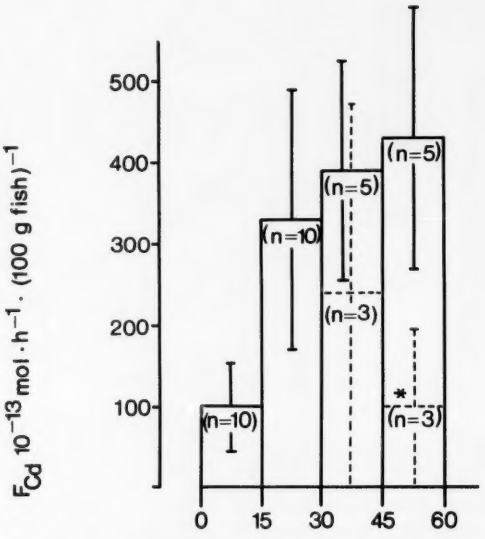

FIG. 4. Influx of cadmium F_{Cd} (mean ± SD), in mol·h^{-1}·(100 g fish)$^{-1}$ through perfused gills of rainbow trout. The concentration of the metal in the water outside the gills is 5×10^{-7} mol·L^{-1} (56 μg·L^{-1}). After 30 min, 2.9×10^{-5} mol·L^{-1} EDNTA is added to the water. The solid lined columns show the influx before the EDNTA addition, the dotted lines after addition (*$P < 0.05$).

At a concentration of 1.0×10^{-5} mol·L^{-1}, EDNTA, a chelating agent similar to EDTA and NTA decreased the free cadmium ion concentration in the water containing 5×10^{-7} mol·L^{-1} Cd below the detection limit (5×10^{-8} mol·L^{-1}) when measured with a cadmium-selective electrode (P. Pärt, University of Uppsala, Institute of Zoophysiology, S-751 22 Uppsala, Sweden, unpublished data). In the present perfusion system, addition of 2.9×10^{-5} mol·L^{-1} EDNTA to the external water containing 5×10^{-7} mol·L^{-1} Cd decreased cadmium transfer through the gills (Fig. 4). Thus, the transfer of cadmium in perfused gills seems to be at least partly dependent on the free cadmium ion concentration outside the gills. The similarity in this respect between the perfused gill preparation and the situation in the whole animal supports the idea that perfused gills can be used in studies of heavy metal availability to fish.

General Discussion and Conclusions

In the entire organism, the metal content is a result of uptake, metabolism, and excretion. Environmental factors affect these processes in different ways. With this in mind the approach to use in vitro techniques to study the uptake process separately is well justified.

Two main kinds of perfused gill preparations have been described for in vitro studies of gill function: perfusion of a single gill arch (Richards and Fromm 1969; Rankin and Maetz 1971; Shuttleworth 1972) and perfusion of the whole basket as in the head perfusion technique (Payan and Matty 1975).

In the present study the head perfusion technique was chosen because it offers a larger gill area to the surrounding water, which is especially important when substances with such a low transfer rate as cadmium are being studied.

To our knowledge the uptake rate of cadmium through the gills of salmonid fishes has not been measured in vivo. Available data from other fish species are not directly comparable to our data because of differences in water quality, temperature, time points of measurements, Cd concentrations, etc. However, a rough comparison indicates that the uptake rate of cadmium in the perfused gills is lower than in vivo (Eisler 1974; Pascoe and Matty 1977).

The objective of the present study has not been to measure the absolute cadmium flux through the gills but to identify factors that influence it. The former question can be settled only by measurements in vivo under natural conditions. However, the flux of cadmium in the isolated gills in this study has been shown to react in a similar way to changes in the chemical form of the metal, e.g. in the presence of chelators, as in whole fish experiments. Consequently the gill perfusion technique seems to be a useful tool in this field in environmental research although there are still methodological problems to solve. Further research is in progress.

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Establishment of Walking Catfish (*Clarias batrachus*) Cell Lines and Development of a Channel Catfish (*Ictalurus punctatus*)Virus Vaccine

EDWARD J. NOGA[1] AND JAMES X. HARTMANN

Department of Biological Sciences, Florida Atlantic University, Boca Raton, FL 33431, USA

NOGA, E. J., AND J. X. HARTMANN. 1981. Establishment of walking catfish (*Clarias batrachus*) cell lines and development of a channel catfish (*Ictalurus punctatus*) virus vaccine. Can. J. Fish. Aquat. Sci. 38: 925–930.

Cell lines from apparently normal gill, gonad, and kidney tissue of adult walking catfish (*Clarias batrachus*) were established and have been subcultured 75, 80, and 95 times, respectively. The cells were propagated in a modified Ham's F-12 medium at 25°C but are capable of growth at 37°C. The gill and kidney cells are the first to have been established from these tissues in fish. The kidney cell line is fibroblastic, the gonad is a mixed epithelioid–fibroblastic type, and the gill is pleomorphic. All three cell lines are susceptible to channel catfish virus (CCV), exhibiting cell fusion and other marked cytopathic effects. A live attenuated vaccine strain of CCV was produced by repeated passage of a virulent strain of CCV in kidney cell cultures. The vaccine strain is less virulent than the wild-type CCV and protects fingerling catfish against challenge with wild-type virus.

Key words: channel catfish virus, *Clarias batrachus*, fish cell cultures, vaccine

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Des lignées cellulaires provenant de tissus de branchies, de gonades et de reins apparemment normaux de *Clarias batrachus* ont été établies et sous-cultivées 75, 80 et 95 fois, respectivement. On s'est servi d'un milieu de culture F-12 de Ham modifié à 25°C, bien que les cellules puissent croître à 37°C. Les cellules branchiales et rénales sont les premières à avoir été cultivées à partir de tissus provenant de poissons. La lignée de cellules rénales est fibroblastique, celle de la gonade est du type épithéloïde–fibroblastique mixte, alors que celle de la branchie est pléomorphe. Les trois lignées cellulaires sont sensibles au virus de la barbue de rivière (CCV) : il y a fusion cellulaire et autres effets cytopathiques marqués. Par passages répétés d'une lignée virulente de CCV dans des cultures de cellules du rein, nous avons produit un vaccin atténué de CCV. La lignée du vaccin est moins virulente que le type sauvage de CCV et protège les jeunes *Clarias* contre les attaques du virus sauvage.

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CHANNEL catfish virus (CCV) disease is one of the most serious fish diseases in North America (Thompson et al. 1973). A herpes type virus, it causes the only known virus disease of warmwater fishes in North America that results in serious losses. Since its first isolation in Alabama (Fijan et al. 1970), it has been reported in 11 additional states. Mortality may reach 100% (Plumb 1974) and losses due to individual epizootics have exceeded $500 000 (McCraren 1973).

Our report describes the establishment of three new cell lines, including the first to be established from fish kidney and gill. The kidney cell line provides a means for producing an attenuated strain of CCV that can prevent the disease through active immunization. The cell lines were derived from the walking catfish, *Clarias batrachus*, an exotic species in Florida.

Material and Methods

Fish used for the initiation of cell cultures weighed at least 50 g. A modification of the procedure described by McKenzie and Stephenson (1973) was used to establish the kidney and gonad primary cultures (Noga 1979). Organs were cut into 1-mm^3 fragments, washed with calcium- and magnesium-free saline solution (CMF), resuspended in 0.25% trypsin for ~30 min, then the trypsin solution was decanted and the fragments plated. The tissues generally adhered to the flask surface within seconds and culture medium was then added. All procedures were performed at room temperature.

A different procedure was used for culturing gill tissues prior to the development of the above-improved method.

[1]Present address: College of Veterinary Medicine, University of Florida, Gainesville, FL 32610, USA.

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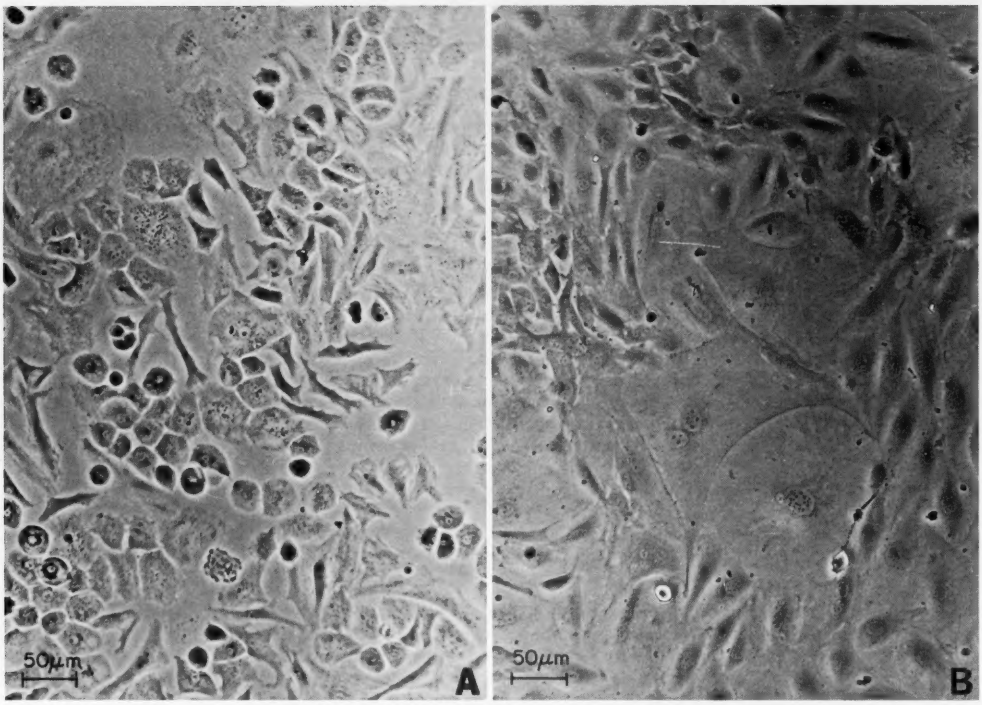

FIG. 1. Walking catfish (*Clarias batrachus*) cell cultures. (A) Gill cells at 30th passage showing pleomorphic appearance; (B) gonad cells at 50th passage showing mixed epithelioid–fibroblastic appearance. Bar = 50 μm.

Opercula were removed and the excised gill fragments were washed twice with four volumes of CMF to remove blood and debris. The fluid was replaced with culture medium containing penicillin G (400 units/mL), streptomycin sulfare (400 μg/mL), and amphotericin B (10 μg/mL), and the tissue was agitated on a rotary shaker at 110 rpm for 24 h at room temperature. The medium was changed and the procedure repeated. The arches were then cut into small pieces and placed in a culture flask which was placed upright for about 1.5 h to drain the medium from the fragments. The fragments were then flooded with culture medium and incubated at 25°C.

Medium for *Clarias* cell cultures consisted of a modified Ham's F-12 in which all components were present at 52% of the standard concentration. Additionally, the medium contained 23 mmol/L HEPES buffer, 25 mmol/L sodium bicarbonate, 9% fetal bovine serum, and the antibiotics penicillin G (200 units/mL), streptomycin sulfate (200 μg/mL), and amphotericin B (5 μg/mL). The pH of the medium was 7.4.

Medium was changed on the primary cultures once or twice a week. When the cultures became confluent, cells were removed by trypsinization. The cell sheets were washed twice with CMF for 5 min each, after which 0.5 mL of 0.25% trypsin was added to each culture (i.e. usually 10% of the original culture volume). After cells had detached, a sample was removed and fresh medium was added. During the first trypsinization of the primaries no cells were taken out of the flask; rather, fresh medium was added to acclimate the cultures to enzymatic treatment while not reducing the cell density. Subsequently, confluent cultures were subcultivated at a 1:2 ratio every 4–7 d.

To confirm that the cell lines were of clariid origin, we used the cytotoxic antibody test of Greene and Charney (1973). Antiserum was absorbed 5 times at 37°C with an equal volume of packed brown bull head (*Ictalurus nebulosus*) (BB) cells.

To study the viral susceptibility of the *Clarias* cells, we propagated CCV in BB cells. Culture medium for the BB cells consisted of minimum essential medium with Hanks' balanced salt solution (HBSS) supplemented with 4 mmol/L sodium bicarbonate and 9% fetal bovine serum. Antibiotic concentrations were half those used for the *Clarias* cells. *Clarias* cells used for experiments with CCV were seeded into 13 × 90 mm Leighton tubes at the rate of 5×10^5 cells per tube. When the monolayers were 90–100% confluent, they were infected with CCV and incubated at 25°C.

Attenuation of a virulent strain of the virus was accomplished by following the procedure of Sabin et al. (1954). A 0.1-mL sample of the Auburn strain virus (Dr John Plumb, Auburn University) (CCV-5), which had been previously passaged about 35 times in BB cells, was inoculated into a

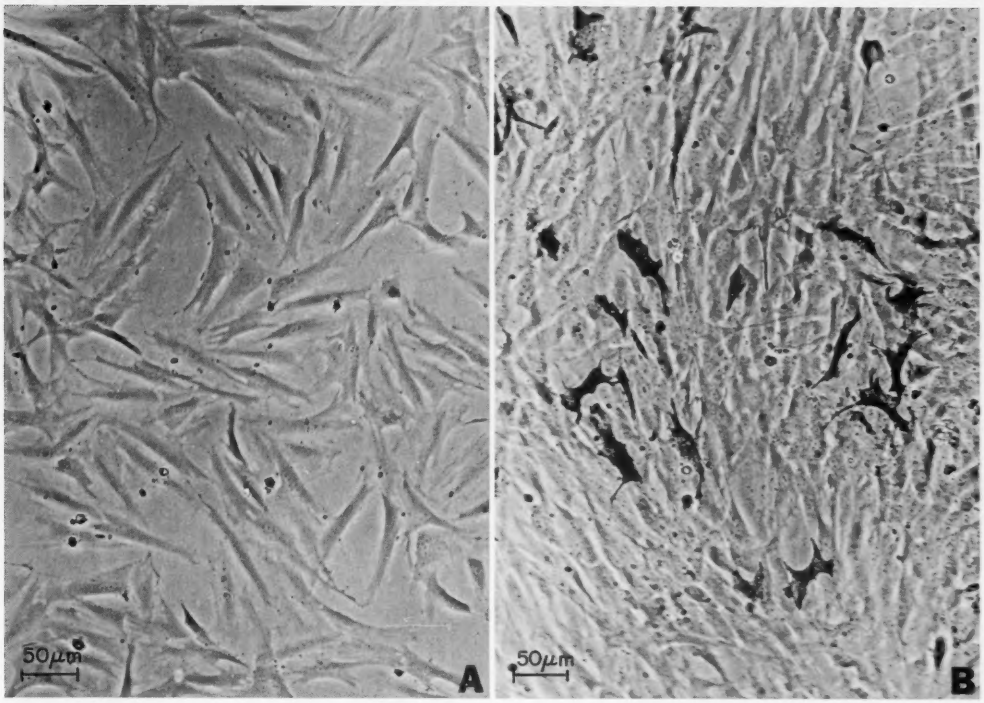

FIG. 2. Walking catfish (*Clarias batrachus*) kidney cells. (A) Fibroblastic appearance at 60th passage; (B) confluent culture at 45th passage containing numerous pigmented cells (arrows). Bar = 50 μm.

Leighton tube containing a kidney cell monolayer and 1 mL of medium. When 90–100% of the monolayer showed viral effects, 0.1 mL of fluid was removed, inoculated onto a new culture, and the process repeated. After the 54th passage, this virus material was diluted to the end point of its infectivity 3 times, as determined by the method of Rovozzo and Burke (1973). It was then passaged an additional 3 times. This virus material (V60) was tested for virulence and used as a vaccine to determine if it would confer protective immunity.

The pathogenicity of the virus strains was tested by injecting fish intraperitoneally with 0.01–0.10 mL of test suspension. Three virus strains were tested: CCV-5, V60, and WT, a wild-type strain isolated from an epizootic of CCV in Florida and passaged in BB cells 3 times. Fish were injected with 10-fold dilutions ov virus suspension. Five fish were used for each dilution. Controls were injected with HBSS. Fish were maintained at 27–29°C, examined daily, and observed for 3 wk.

To ascertain whether protection was conveyed by immunization with the vaccine strain, 20 fish were challenged intraperitoneally (5 fish in four experiments) with wild-type (WT) virus 3 wk after vaccination with V60 at 27–29°C. An equal number of control fish previously injected only with HBSS were also challenged. Deaths were tallied for 3 wk while maintaining fish at 27–29°C.

Results

A pleomorphic cell line from gill tissue (designated G1B) took over 2 mo from initiation to establishment. It has been passaged in excess of 70 times (Fig. 1A).

Using the modified trypsinization technique (Noga 1979) extensive cell migration from explants of kidney and gonad cells occurred in 2–3 d and monolayers resulted within 2 wk. From these primary cultures, two continuous cell lines were established: GD1I (gonad) and K1K (kidney). GD1I, presently in its 80th passage, is a mixed epithelioid–fibroblastic line (Fig. 1B). K1K is a fibroblastic cell line presently in its 95th passage (Fig. 2A). During its first 50 passages, some pigmented cells were presented (Fig. 2B).

Treatment of the three cell lines with anti-*Clarias* serum resulted in 90–95% cytotoxicity for *Clarias* cells with minimal (<10%) activity toward BB cells. The latter is the only other cell line maintained in our laboratory.

The inoculation of *Clarias* cell cultures with CCV resulted in a distinctive transmissible cytopathic reaction. The outstanding characteristic was cell fusion with resultant syncytia. Foci of fused cells progressively spread with time to encompass the entire monolayer, forming a syncytial network containing clusters of nuclei scattered throughout (Fig. 3A). Cytoplasmic blebs were more common in the infected cultures.

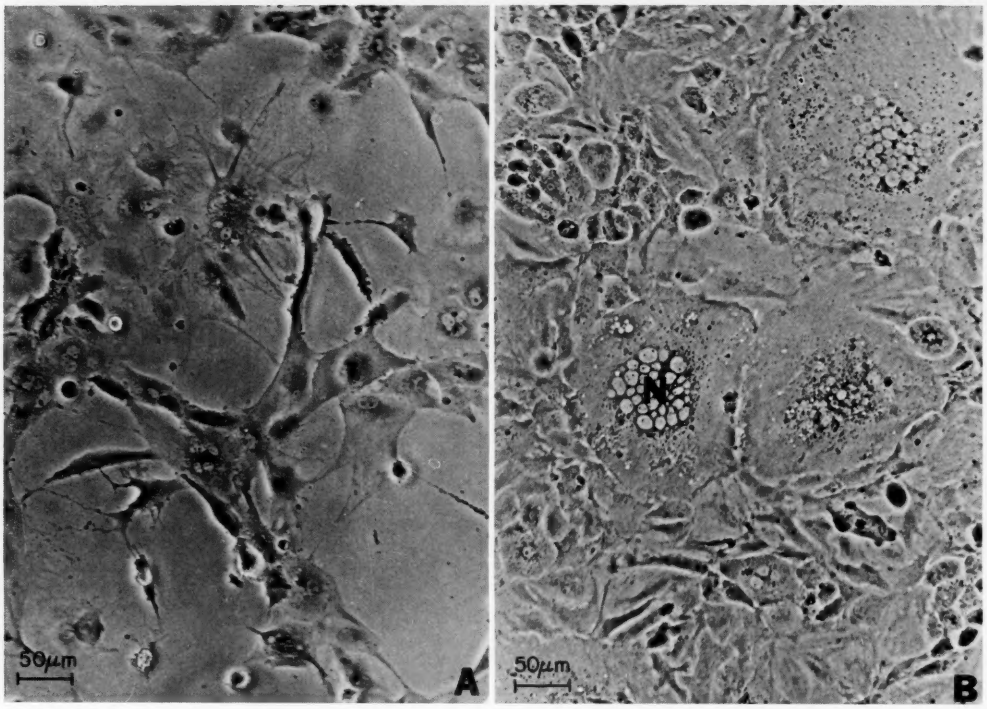

FIG. 3. Walking catfish (*Clarias batrachus*) cells infected with CCV. (A) Gonad cells forming a syncytial network with clusters of nuclei (arrows); (B) gill cells with early stage of infection with well-defined syncytia containing central collection of nuclei (N). Bar = 50 μm.

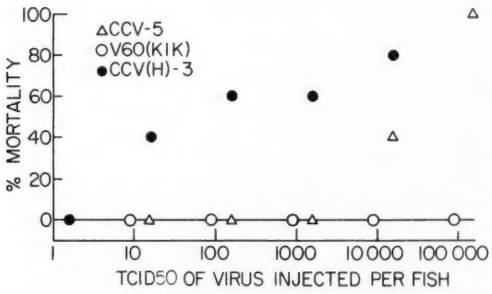

FIG. 4. Pathogenicity of strains of channel catfish virus. (●) WT = wild-type strain isolated from an outbreak of CCV in Florida and passaged 3 times in BB cells; (△) CCV-5 = auburn strain of CCV passaged 35 times in BB cells; (○) V60 = CCV-5 of CCV passaged 60 times in walking catfish kidney cell cultures.

The progressive fusion reaction was most easily witnessed in G1B due to the culture's flat, platelike morphology (Fig. 3B). Vacuoles of variable sizes were a feature of CCV infection in all three cell lines. Controls contained almost no vacuoles.

In G1B cultures, the degree of cell destruction was dependent upon the amount of virus inoculum. If the multiplicity of infection was too low, foci of syncytia would appear but only a portion of the monolayer was destroyed. Sometimes this destroyed area was recolonized by healthy cells after the infective focus had completely degenerated. In K1K and GD1I, complete cell destruction always occurred, irrespective of the virus level with which a culture was infected.

Auburn strain CCV (CCV-5) caused 100% mortality at 100 000 TCID50 units per fish (Fig. 4). The LD50 of CCV-5 was 23 000 TCID50. After 60 passages in *Clarias* kidney cells the virulence of the virus was reduced. No deaths resulted from injection of V60 even though fish were challenged with up to 90 000 TCID50. The LD50 of WT was 585 TCID50. All fish injected with HBSS survived. Ten percent of the V60-vaccinated fish succumbed to challenge with WT virus whereas 100% of the unvaccinated controls died. Significant protection was afforded vaccinated fish challenged with a lethal dose of WT virus (chi-square test, $P < 0.005$).

Discussion

Three *Clarias* cell lines were established: K1K (kidney), GD1I (gonad), and G1B (gill). Prior to the establishment of these *Clarias* cultures, cell cultures derived from the ictalurid

fishes had been the only cells known to be susceptible to channel catfish virus (Wolf and Darlington 1971). Aside from BB and CCO, another ictalurid cell line (Bowser and Plumb 1980), K1K and GD1I are to date the only established cell lines known to support growth of CCV.

The K1K cell line possessed pigmentation through its first 50 passages. Whereas pigmented cell lines have been derived from neoplastic tissues of several mammalian species, few long-term cultures of melanocytes derived from normal tissues of any animal species have been established. *Clarias* kidney cells may be a useful tool in differentiation studies and for comparison with malignant melanotic cells. Another interesting property of both K1K and GD1I is their ability to survive and grow at 37°C (Noga 1977). The upper lethal limit of BB cells is 34°C whereas CCO has been demonstrated to grow up to 35°C. It has been assumed that CCV cannot replicate at 37°C (Wolf 1975); the use of *Clarias* cell lines may allow replication studies with CCV at this higher temperature.

To our knowledge, K1K and G1B are the first continuous fish kidney and gill cell lines to have been established (Wolf and Mann 1980). Whether these cells retain any biochemical functions specific to their organ of origin was not determined. Thus, the kidney cells, being purely fibroblastic, may be of connective tissue origin and not derived from kidney parenchyma.

Channel catfish fingerlings, parenterally immunized with a *Clarias* cell culture attenuated strain of CCV, were protected from parenteral challenge with a lethal dose of wild-type virus. Although these data demonstrate significant protection, further studies involving oral immunization trials will have to be performed to ascertain the feasibility of using this vaccine as an immunizing agent on a commercial scale because injection of individual fish is not economically feasible.

The use of a live vaccine may be a useful alternative to serological or virological surveillance for the effective control of CCV. Unlike other fish viral diseases such as infectious hematopoietic necrosis, infectious pancreatic necrosis, and viral hemorrhagic septicemia of salmonids, in which asymptomatic carriers exist (Amend 1976), CCV cannot be isolated except during an active epizootic (Plumb 1971). Thus asymptomatic carriers may continue to shed the virus and expose susceptibles. Although detection of neutralizing antibody in adult channel catfish has been studied as a means of detecting carriers, it is an indirect method and as yet cannot be relied upon to suggest a carrier state but is indicative of previous exposure to the virus (Plumb 1973, 1974).

An immune response to injection of viable CCV has also been observed by Heartwell (1975), who showed that channel catfish were capable of responding to CCV 1 wk after primary injection and peak antibody titers occurred in 60 d at 27–28°C. This was similar to the time required for peak titers in Plumb's (1973) studies done at 22–24°C. It is also noted that Plumb (1973) could not detect any immune response after two injections of a heat-killed virus. The response of channel catfish to vesicular stomatitis virus, a nonpathogenic virus, was similarly of low titer and declined rapidly (McGlamery et al. 1971). Whereas the previous studies were performed with subadult and adult channel catfish, our study has demonstrated that young channel catfish are also immunocompetent to CCV. This was true for the response of fingerlings to a particulate antigen, *Chondrococcus columnarus* (Anon. 1971). Fryer et al. (1976) similarly found that juvenile salmon were capable of resisting infection to IHN virus after being immunized with an attenuated strain of the virus.

Acknowledgments

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Fate of Kepone and Mirex in a Model Aquatic Environment: Sediment, Fish, and Diet

DONALD R. SKAAR[1]

School of Forestry, Fisheries and Wildlife, University of Missouri, Columbia, MO 65211, USA

B. THOMAS JOHNSON

Columbia National Fisheries Research Laboratory, U.S. Department of the Interior, Fish and Wildlife Service, Columbia, MO 65201, USA

JOHN R. JONES

School of Forestry, Fisheries and Wildlife, University of Missouri, Columbia, MO 65211, USA

AND JAMES N. HUCKINS

Columbia National Fisheries Research Laboratory, U.S. Department of the Interior, Fish and Wildlife Service, Columbia, MO 65201, USA

SKAAR, D. R., B. T. JOHNSON, J. R. JONES, AND J. N. HUCKINS. 1981. Fate of Kepone and mirex in a model aquatic environment: sediment, fish, and diet, Can. J. Fish. Aquat. Sci. 38: 931–938.

Laboratory studies of freshwater sediment and a daphnid–bluegill (*Daphnia magna–Lepomis macrochirus*) food chain indicated that the organochlorine insecticides Kepone and mirex resist microbial degradation, accumulate and resist metabolism in fish, and move through a food chain. We found no direct evidence of degradation of either compound after 56 d of incubation in freshwater sediments under aerobic or anaerobic conditions. Furthermore, we found no metabolism or co-metabolism of ^{14}C-labeled Kepone in sediments that had long-term preexposure to Kepone and by-products nor did we detect a selective process occurring in the sediments that changed the capacity of the autochthonous microbiota to metabolize Kepone. After 28 d of exposure to ^{14}C-labeled Kepone or mirex in water and natural food, bluegills accumulated Kepone and mirex equal to 10 606 and 12 274 times the respective exposure concentrations. Elimination patterns were dissimilar: mirex tended to persist in fish tissue but Kepone did not. Neither Kepone nor mirex was metabolized by bluegills. Retention of mirex introduced into bluegills by daphnids was about 3 times greater than that of Kepone. Accumulation of Kepone and mirex by bluegills was about half that of DDT.

Key words: Kepone, mirex, degradation, freshwater sediment, accumulation, elimination, bluegills, daphnids, residue dynamics, food chain, accumulation index

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D'après des études en laboratoire de sédiment d'eau douce et d'une chaîne alimentaire constituée par une daphnie (*Daphnia magna*) et du crapet arlequin (*Lepomis macrochirus*), les insecticides organochlorés Kepone et mirex résistent à la dégradation microbienne, s'accumulent dans le poisson, y résistent à la métabolisation et se transmettent le long de la chaîne alimentaire. Nous n'avons trouvé aucune preuve directe de la dégradation des deux composés après 56 jours d'incubation dans des sédiments d'eau douce, en conditions aérobies ou anaérobies. De plus, nous n'avons observé aucune métabolisation ni cométabolisation de la Kepone marquée au ^{14}C dans les sédiments qui avaient été préalablement exposés à long terme à la Kepone et à ses sous-produits et nous n'avons pas décelé non plus de processus sélectifs

[1]Present address: Department of Biology, Montana State University, Bozeman, MT 59717, USA.

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qui ont modifié l'aptitude des microbes indigènes des sédiments à métaboliser cet insecticide. Après 28 jours d'exposition à la Kepone ou au mirex marqués au ^{14}C, dans l'eau ou la nourriture naturelle, les crapets arlequins ont concentré la Kepone et le mirex, 10 606 et 12 274 fois leurs concentrations respectives de départ. Les formes d'élimination différaient: le mirex tendait à persister dans les tissus, contrairement à la Kepone. Les crapets n'ont métabolisé ni la Kepone ni le mirex. La rétention du mirex absorbé par les arlequins dans les daphnies a été 3 fois supérieure à celle de la Kepone. L'accumulation de la Kepone et du mirex par les crapets était inférieure de moitié à celle du DDT.

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KEPONE and mirex were found in water, sediments, and biota of freshwater and marine ecosystems (Saleh et al. 1978; Kaiser 1978; Borthwick et al. 1973) at levels disproportionately high to the small quantities released into the environment (Livingston 1978). The two organochlorine insecticides are structurally similar[2] (Fig. 1) but differ in chemical properties and in behavior within aquatic ecosystems (Livingston 1978).

Kepone has had only limited use in the United States as cockroach bait (Suta 1977); most of the compound has been synthesized for export. Therefore, the major sources of environmental contamination are at the manufacturing sites (Livingston 1978). In 1975 it was discovered that Life Sciences Product Company, Hopewell, Virginia, discharged about 45 000 kg of Kepone and by-products into water that eventually entered Chesapeake Bay, contaminating about 380 000 m^2 of sediment (Gregory 1976; Environmental Protection Agency 1976) and thus threatening an important estuarine fishery.

Mirex has been used extensively as an insecticide and fire retardant (Farm Chemical Handbook 1975). Since 1967 it has been sprayed at a rate of 4.2–8.6 g/ha on over 400 000 ha/yr to control the fire ant (*Solenopsis* sp.) in 11 southeastern states (Suta 1977). It was later detected in stream water and in estuarine biota (Baetcke et al. 1972; Borthwick et al. 1973; Pritchard et al. 1973; Tagatz et al. 1975).

We conducted laboratory studies to assess the potential hazard of these chemical contaminants due to their persistence and accumulation in the aquatic environment. We report on the ability of freshwater sediment to degrade Kepone or mirex and the ability of a freshwater fish to accumulate, metabolize, and eliminate Kepone or mirex from water or natural food.

Materials and Methods

^{14}C-labeled Kepone (UL, 1.158 MBq/mg) and ^{14}C-labeled mirex (UL, 0.492 MBq/mg) were purchased from Pathfinder Laboratories, Inc., St. Louis, Missouri. Purity exceeded 99% as determined by gas–liquid and thin-layer chromatography (TLC).

[2]Kepone (decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]-pentalen-2-one) and mirex (dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalene) are both synthesized from hexachlorocyclopentadiene (Fig. 1). They differ in that two chlorine atoms in mirex are replaced with a double-bonded oxygen atom in Kepone; Kepone is about 2000 times more soluble in water than mirex (Livingston 1978).

Freshwater Sediments and Microbial Degradation

Sediment samples were taken from Little Dixie Lake, Missouri, an 83-ha impoundment located in an agricultural watershed 16 km east of Columbia. Chemical characteristics of the water are similar to those of other surface waters in central Missouri (Jones 1977). The lake is slightly eutrophic; alkalinity is entirely bicarbonate (Heman et al. 1969); organic matter content of hydrosoil samples averaged 1.6% (dry weight). Samples were collected in April and October 1977 and April 1978 from the littoral zone (about 1.5 m in depth) with an Ekman dredge. An electron-capture gas chromatographic analysis of the sediment revealed no detectable Kepone or mirex (minimum detectable limit, 0.02 μg/g).

Sediment samples from Bailey Creek, Virginia, were obtained with a hand shovel in October 1977. This tributary of the James River drains several industrial sites and receives the effluent from the Hopewell municipal sewage plant. It has been contaminated with Kepone and Kepone by-products for years (Gregory 1976).

Possible microbial degradation of Kepone and mirex was tested in the upper 3–5 cm of sediment under aerobic and anaerobic conditions. For the Kepone microbial degradation studies only, we selected two sediments: one with known extensive continuous exposure to Kepone (Bailey Creek) and one without known or evident Kepone contamination (Little Dixie Lake).

We placed 10 g (wet weight) of sediment in a 50-mL Delong flask for aerobic studies or in a 30-mL screw-top glass tube for anaerobic studies, and added 20 mL of water from either Baily Creek (Kepone study only) or Little Dixie Lake. The contents were inoculated with 300 μL of acetone containing 3 μg of either ^{14}C-labeled Kepone or mirex (final concentration 0.1 μg/g). Control samples were autoclaved for 20 min at 121°C under 1.05 kg/cm^2 steam pressure; to ensure sterility 0.75 g of mercuric chloride was added to each container. All samples were incubated at 22°C in the dark, the aerobic group on a rotary shaker and the anaerobic group in a torsion jar evacuated under vacuum and overlaid with nitrogen. Samples taken in triplicate at intervals of 7, 14, 21, 28, and 56 d were frozen for later determination of degradation products of either Kepone or mirex by TLC autoradiography and $^{14}CO_2$ radiorespirometry (Johnson and Lulves 1975).

For TLC analyses, sediment samples were thawed to room temperature for extraction and inoculated with 2 mL of saturated KOH and 1.0 g of copper powder to remove sulfur. The mixture was acidified with 0.1 mol/L HCl and extracted with three 15-mL portions of ethyl acetate. The organic layer was

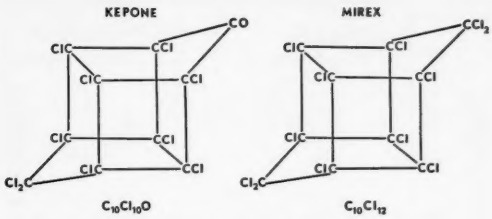

FIG. 1. Chemical structures of Kepone and mirex.

removed by centrifugation. The ethyl acetate extractions were combined, concentrated, and purified on an AMOCO px-21 carbon/foam column (Huckins et al. 1978) to remove interfering biological material. The ^{14}C-labeled Kepone extracts were eluted from the carbon/foam column with sequential washes: 50 mL of petroleum ether, 100 mL of ethyl acetate, and 50 mL of methanol; ^{14}C-labeled eluates of Kepone were spotted on a silica gel plate (0.25 mm in thickness) and developed 3 times in a 4:1 (v/v) mixture of benzene and acetone. Autoradiograms were made by exposing the TLC plate in the dark to Kodak no-screen X-ray film for 7 d. R_f values and tentative identification of separated extracts were made by comparing cochromatographed nonlabeled standards with the radiogram. Residues of ^{14}C-labeled mirex were extracted from sediment as described for Kepone. The TLC plates were developed in 5% diethyl ether in petroleum ether (v/v).

EVOLUTION OF $^{14}CO_2$ FROM SEDIMENTS

Sediments of Little Dixie Lake were incubated with labeled Kepone and the effluent gas (air in the aerobic test and nitrogen gas in the anaerobic test) was measured with a radiorespirometer (Johnson and Lulves 1975). The gas was bubbled slowly through a trapping solution of monoethanolamine–ethylene glycol (3:7, v/v). Samples (1 mL) of the trapping solution were added to 15 mL of a 1:1 mixture (v/v) of methanol and Fluoralloy (Beckman Instrument Co.). In triplicate these samples were quantified using a Beckman LS-200 liquid scintillation counter (LSC) and corrected for quench and background radiation. The ^{14}C counting efficiency was 85 ± 5%.

Evolution of $^{14}CO_2$ from sediments of Little Dixie Lake was measured by a method modified from Gledhill (1975). Sediment was incubated with mirex in 250-mL Erlenmeyer flasks. Glass vials suspended from rubber stoppers in each flask contained 10 mL of trapping solution of monoethanolamine–ethylene glycol (3:7, v/v). Aerobic conditions were maintained by aerating the samples for several minutes semiweekly, and anaerobic conditions by overlaying the sample with gaseous nitrogen. Triplicate 1-mL aliquots were counted by the LSC, as described for Kepone. Anaerobiosis was monitored with methylene blue (Manual of Microbiological Methods 1957).

MODULAR FOOD CHAIN

Residue dynamics of Kepone and mirex were examined in bluegills (*Lepomis macrochirus*) fed daphnids (*Daphnia magna*) according to the methods of Johnson (1974, 1980). Accumulation studies were conducted in an intermittent flow-through diluter system modified after Mount and Brungs (1967) and McAllister et al. (1972). A flow-splitting chamber, modified after Benoit and Puglisi (1973), was used to mix and divide the toxicant for delivery to the exposure tanks (78 L). Photoperiodicity was 16-h light:8-h dark.

Well water (pH of 7.4 and total hardness 270 mg/L as $CaCO_3$) was aerated and maintained at 22 ± 1°C for all experiments. Stock solutions of each of the ^{14}C-labeled toxicants were made up in acetone. We detected no deleterious effect on daphnids or bluegills in control chambers exposed to 0.33 mL/L acetone. Toxicant concentrations in the exposure chambers were monitored 3 times per week by the radiometric method described. We removed 500 mL of water from the exposure chamber and extracted it twice in a separatory funnel with 15 mL of analytical grade methylene chloride. Exactly 5 mL of toluene (scintillation grade) was added to the methylene chloride extract and the mixture was evaporated in a porcelain evaporation dish to 1–2 mL. This volume was transferred with 15 mL of Triton-X–Fluoralloy (6:9, v/v) into scintillation vials. Radioactivity was quantified in a Beckman LS-200 LSC. Corrections were made for extraction efficiency (85% Kepone; 82% mirex) and quench.

Bluegills were obtained from Federal hatcheries and maintained by the methods of Brauhn and Schoettger (1975); daphnids were maintained by the method of Dewey and Parker (1964) at our laboratory.

Residue dynamics of Kepone and mirex in fish were determined after exposure in water and natural food. We placed 40 bluegills (0.5–1.5 g) in each of four 78-L chambers. Three chambers were continuously exposed to a constant water concentration of Kepone (or mirex) at 150 ng/L for 28 d. Daphnids were exposed for 24 h to the same ^{14}C-labeled insecticide in the same tanks within a screened chamber. Fish were fed treated daphnids daily at either 10% (Kepone) or 7.5% (mirex) of their body weight (wet). Five 50-mg samples of daphnids were frozen for residue analyses semiweekly by the radiometric method of Johnson et al. (1971).

Seven fish were removed from each exposure chamber for the determination of total body ^{14}C-labeled residue after 7, 14, 21, and 28 d of exposure to either ^{14}C-labeled Kepone or ^{14}C-labeled mirex. Total body residues were determined by the radiometric method of Johnson (1980) and measured with an LSC. Data were corrected for quench (15 ± 5%) and background radiation. Accumulation factors were determined by dividing the toxicant concentration in the fish by the toxicant concentration in the water.

On day 28, four or five fish from each aquarium (about 15 fish) were frozen for metabolite analyses; the rest were transferred to compound-free water to monitor elimination after 1, 3, 7, 14, 21, and 28 d (4 fish assayed per date). Fish were fed untreated daphnids at the previous rations.

To determine the accumulation of Kepone and mirex from daphnids, we exposed 40 bluegills to compound-free water in the flow-through diluter, as described in the previous experiment. Fish were fed daphnids exposed for 24 h to 400 ng/L Kepone (or mirex) at 10% of their body weights each day. Seven fish were removed for LSC analyses of total body residue at 7, 14, 21, and 28 d.

Data were subjected to statistical analyses at $\alpha = 0.05$ by linear regression, analysis of variance, and multiple means analysis (least significant difference).

Metabolism of Kepone

Analytical methods for Kepone analysis were modified after the work of J. N. Huckins (Columbia National Fisheries Research Laboratory, U.S. Department of the Interior, Fish and Wildlife Service, Columbia, MO, unpublished data). The frozen fish were ground in a Sorvall Omni-mixer, blended with 4 times their weight of anhydrous Na_2SO_4, and poured into an extraction column (29 cm × 2 cm ID). The fish homogenate in the column was washed sequentially with three solutions: 150 mL of 5% diethyl ether in petroleum ether, 150 mL of ethyl acetate–acetone (1:1 v/v), and 50 mL methanol. We concentrated and chromatographed the second eluate on a silica gel column with sequential washes: 50 mL of hexane, 50 mL of 5% methanol in ethyl acetate, and 50 mL of methanol. The second eluate from the silica gel column was concentrated to 2 mL and chromatographed on an Autoprep 1001 gel permeation chromatograph (GPC). The GPC column (30.5 cm × 2.5 cm ID) was packed with 70 g of Sephadex LH-20 (25–100 μm). The solvent system was ethyl acetate–methanol–toluene (80:10:10, v/v/v). We examined fractions containing radioactive residues by gas chromatography (GC), using a Tracor MT-220 or MT-222 equipped with a ^{63}Ni electron capture detector. The GC columns (15.3 cm × 2 mm ID) were packed with 3% OV-7 on 80–100 mesh Chromosorb W HP (w/w). Carrier gas (N_2) flow was 30 mL/min; oven and injector temperatures were set at 190 and 220°C, respectively.

The analysis of mirex was similar to that of Kepone. Fish tissue was extracted in a column with 150 mL of 5% diethyl ether in petroleum ether, followed by 50 mL methanol. The first column eluate was concentrated to about 2 mL and chromatographed on a silica gel column with 43 mL of 0.5% benzene in hexane, 15 mL of 4% ethyl acetate in benzene, and 25 mL methanol. The first eluate of the silica gel column was concentrated to 2 mL and chromatographed on GPC with a column packed with 50 g Bio-Beads S-X3 (200–400 mesh) using a mixture of cyclohexane–methylene chloride (85:15, v/v). Fractions containing radioactive residues were analyzed for percent recovery of mirex by GC analysis, as described above.

The recovery of radioactive residues from fish for both ^{14}C-labeled compounds was verified by complete combustion of whole body samples and measurement of $^{14}CO_2$ after completion of solvent extractions.

Results and Discussion

Toxicant Degradation in Sediments

We found no direct evidence that Kepone or mirex was degraded in these freshwater sediments under either aerobic or anaerobic conditions. After 56 d of incubation we could detect no significant ($P > 0.05$) evidence of either primary or ultimate degradation of either Kepone or mirex. Concentrations ≤10 mg/kg of either Kepone or mirex did not impair respiration in sediments (Johnson 1981).

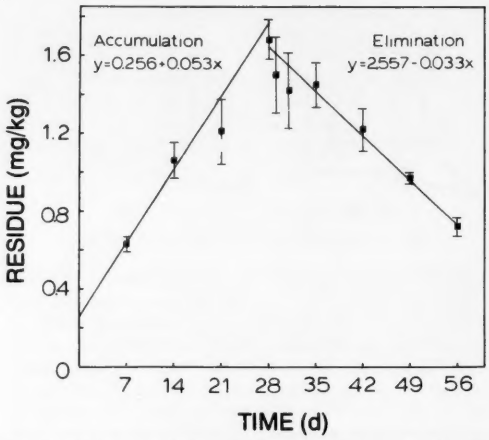

FIG. 2. Mean accumulation and elimination of Kepone by bluegills from food and water over a 56-d period. Vertical bars show standard deviation.

Radiorespirometric and TLC autoradiographic analyses of sediments from Little Dixie Lake (Missouri) and Bailey Creek (Virginia) indicated that no degradation of Kepone had occurred. Recovery of ^{14}C-labeled Kepone from sediments ranged from 75 to 94%, which compared favorably with recoveries of 83–103% from spiked control sediments. Kepone was tightly bound to the sediment and required vigorous treatment to effect chemical extraction. Although sediments in the Bailey Creek area had been contaminated with Kepone and its by-products for years (Gregory 1976), this preexposure did not increase the microbial capacity to degrade Kepone. We detected no natural selective process toward enzymatic induction with the autochthonous microbiota. The Bailey Creek sediments exhibited the same inability to degrade Kepone. We were thus unable to corroborate the findings reported by Orndorff and Colwell (1979) of Kepone degradation in the Bailey Creek sediments.

We did detect significantly higher ($P > 0.05$) $^{14}CO_2$ evolution from aerobic sediments treated with ^{14}C-labeled mirex in one of four sampling dates (day 7). This difference represented 0.9% of the total labeled mirex inoculated and occurred only during the 1st week. We suspect a low-level ^{14}C contaminant rather than a metabolite of mirex. No significant difference ($P < 0.05$) was detected between treated and control sediments under anaerobic conditions. Autoradiograms revealed only mirex. These results support the findings of Jones and Hodges (1974), who found no evidence of biological degradation of mirex in soil.

Modular Food Chain

After 28 d of exposure to ^{14}C-labeled Kepone in food (0.046 ± 0.01 mg/kg) and in water (158 ± 13 ng/L), bluegills contained a mean total body residue of 1.68 ± 0.11 mg/kg Kepone. A plot of body residue versus time indicated that Kepone accumulation from water and food was

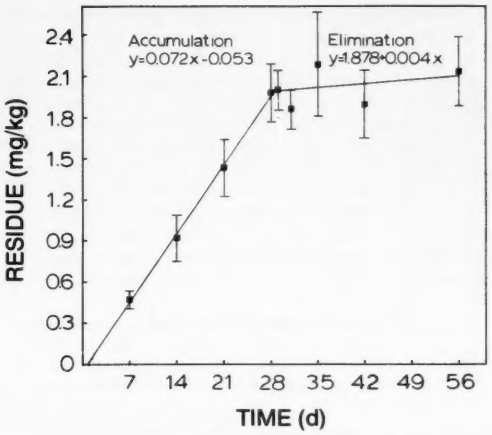

FIG. 3. Mean accumulation and elimination of mirex by bluegills from food and water over a 56-d period. Vertical bars show standard deviation.

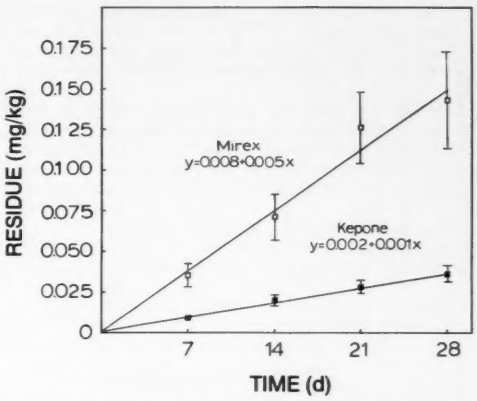

FIG. 4. Mean accumulation of Kepone and mirex by bluegills from food (*Daphnia*) over a 28-d period. Vertical bars show standard deviation.

constant ($r = 0.99$) between 7 and 28 d. The accumulation factor (AF = concentration in fish divided by concentration in water) was 10 606. The equilibrium concentration of ^{14}C-labeled Kepone in bluegills did not appear to be approached during the 28 d of continuous exposure (Fig. 2). However, determination of the theoretical accumulation equilibrium factor (after Branson et al. 1975) suggests that bluegills were approaching steady state. Estimation of the rate constant for accumulation and elimination and the theoretical accumulation factor of Kepone at equilibrium was calaculated from rate constants modified after the method of Branson et al. (1975) from the equation:

$$C_f / C_w = k_1 / k_2 = K_{cf}$$

where C_f = concentration in fish, C_w = concentration in water, k_1 and k_2 are rate constants for accumulation and elimination, and K_{cf} = the accumulation factor at equilibrium. The estimated rate constant for accumulation and elimination for Kepone was $15.6 \cdot h^{-1}$ and $0.0012 \cdot h^{-1}$. The K_{cf} value of 13 000 was slightly higher than the 10 606 accumulation factor we determined after 28 d of exposure to Kepone. These values obtained for bluegills agree with the accumulation factor reported by Bahner et al. (1977) for young estuarine sheepshead (*Archosasgus probatocephalus*) (exposure period, water concentration, and fish size were similar).

Elimination of Kepone by bluegills in Kepone-free water followed a decreasing, linear pattern described by the equation: $y = 2.5 - 0.03x$ ($r = 0.85$). The estimated half-life of Kepone in bluegills was 25 d. Similarly, Bahner et al. (1977) reported that elimination of Kepone from crustaceans and estuarine fish was low, body residues decreasing 30–60% in 24–28 d.

After 28 d of exposure to ^{14}C-labeled mirex in food (0.24 ± 0.05 mg/kg) and water (161 ± 11 ng/L), bluegills contained a mean total body residue of 1.98 ± 0.2 mg/kg. When these total body residues were plotted against time (Fig. 3), the accumulation of mirex by fish tended to be linear ($r = 0.96$). Equilibrium had not been reached after 28 d of continuous exposure to mirex (Fig. 3). This accumulation factor for mirex (12 274) was about 60 times greater than that reported by Metcalf et al. (1973) in a static, multicomponent model ecosystem. No significant ($P > 0.05$) elimination of mirex by bluegills was detected after 28 d in mirex-free water (Fig. 3).

Bluegills accumulated ^{14}C-labeled Kepone or ^{14}C-labeled mirex from Kepone- or mirex-free water by consuming live daphnids that had previously been exposed to the two insecticides. During separate 28-d feeding experiments, bluegills accumulated 0.036 ± 0.005 mg/kg Kepone and 0.143 ± 0.29 mg/kg mirex from live daphnids containing a mean residue of 0.078 ± 0.020 mg/kg for Kepone and 0.125 ± 0.037 mg/kg for mirex, respectively (Fig. 4). A plot of the data indicated that the accumulation of Kepone and mirex from food was increasing and linear over time: Kepone, $r = 0.91$; mirex, $r = 0.89$ (Fig. 4). Bluegills tended to accumulate more mirex than Kepone from ingested live food. The food transfer factor (consumer residue per food residue (w/w)) was 0.45 for Kepone and 1.14 for mirex. Calculations[3] of the total percent of Kepone and mirex retained by the bluegills indicated that after 28 d, 34% of the mirex and 14% of the Kepone introduced in the daphnids remained in the fish.

[3]The bluegills were fed daily an average of 0.87 g live daphnids which contained 0.78 μg/g Kepone; thus the total Kepone introduced was 0.190 μg per daphnid. All daphnids were rapidly consumed. After 28 feedings the average fish weighed 0.754 g and contained 0.036 μg/g Kepone; therefore the total Kepone retained was 0.027 μg per fish. Percent retention of Kepone by bluegills was determined by the equation:

$$\% \text{ retention} = \frac{\text{total residue (consumer)}}{\text{total residue (prey)}} \times 100.$$

Therefore Kepone retention equals $\frac{0.027\ \mu g}{0.190\ \mu g} \times 100 = 14\%$. Mirex values were calculated similarly.

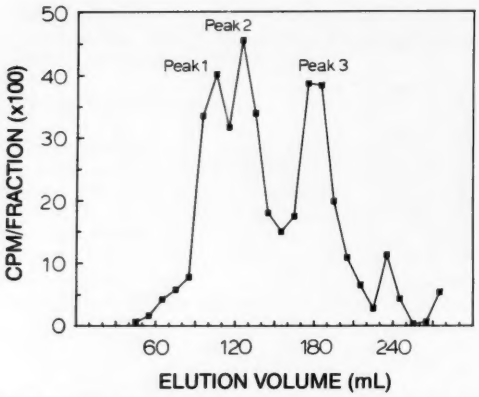

FIG. 5. GPC elution profile of extract from bluegills that had been exposed to Kepone for 28 d before analysis. CPM = counts per minute.

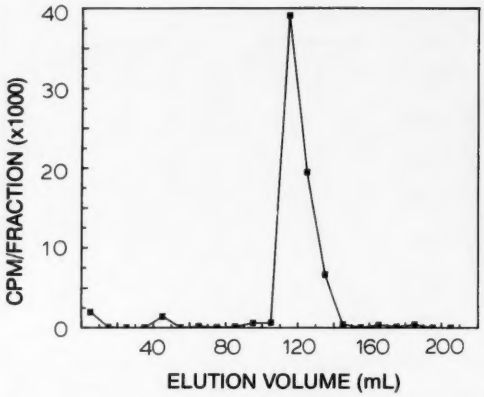

FIG. 6. GPC profile of extract from bluegills that had been exposed to mirex for 28 d before analysis. CPM = counts per minute.

To gain a better perspective of the bioaccumulation hazard of Kepone and mirex to freshwater fish, we calculated an accumulation index value for both compounds. The accumulation index (*AI*), in this instance from food plus water ($f + w$), is a comparison of the accumulation factor of the test chemical ($AF(X)$) with the accumulation factor of p,p'-DDT (AF(DDT)) from a similar source under standard conditions in the protocol described by Johnson (1980). We used the equation:

$$AI_{f+w} = (AF_{f+w}(X))/(AF_{f+w}(\text{DDT})) \times 100.$$

AF_{f+w}(DDT) = 24 323 for bluegills (0.5–1.5 g) after 28 d of continuous exposure to p,p'-DDT (300 ng/L) at 22°C (Johnson 1980). The calculated AI_{f+w} values for Kepone and mirex were 44 and 51. If DDT was given the arbitrary index value of 100, we thus estimated that the propensity for Kepone and mirex to bioaccumulate in bluegills was about half that of DDT.

We found no evidence to suggest that either Kepone or mirex was metabolized by bluegills. However, there was some evidence of Kepone–lipid binding which may or may not be metabolically mediated. A more extensive investigation of metabolic incorporation into lipids has been reported by Huckins (unpublished data).

When extracts from Kepone-treated fish were chromatographed on GPC, the radioactive profiles derived appeared to consist of three main peaks (Fig. 5). Peak 1 (from an elution volume of 90–120 mL) made up 26.7% of the total radioactivity, peak 2 (120–160 mL) 28.3%, and peak 3 (160–220 mL) 31.7%. In analysis of the GPC eluates by GC, 8% of the radioactivity in peak 1, 33% in peak 2, and 80% in peak 3 were identified as Kepone. The minimum detection limit of Kepone was 0.04 μg/g. The lack of an adequate GC response for radioactivity in peak 1 may represent ^{14}C-labeled Kepone–lipid binding products that were probably too large for adequate GC analyses. The concentration of possible lipid-bound ^{14}C-labeled residue in Fig. 5 was 0.7 μg/g. The radioactive residues of peaks 2 and 3 represent Kepone and Kepone derivatives. It appeared that peak 2 was the hemiketal analog of Kepone and methanol, and peak 3 was a combination of Kepone hydrate and an aldol condensation product of acetone and Kepone. The identification of these compounds was based on previous studies (Huckins unpublished data). The Kepone solvent derivatives may be mistaken for degradation products because differences in chromatographic behavior occur during cleanup procedures. Therefore, care must be taken in selecting a solvent for the analysis of Kepone residues.

Although we anticipated possible dechlorination or hydroxylation of Kepone in bluegills, we found none. Acidification of samples did not enhance extraction efficiency. Slight differences of recovery values (8–10%) for Kepone did not necessarily suggest metabolism in the fish.

Chromatography of extracts from mirex-treated fish by GPC yielded only one radioactive peak (Fig. 6). Individual fractions from this peak analyzed by GC showed only mirex. The minimum detection limit of mirex was 0.04 μg/g. About 81% of the radioactive residue from the GPC eluate was recovered and identified as mirex.

The persistence of these compounds in fishes was also shown by Borsetti and Roach (1978), who detected only a trace amount of Kepone metabolites, and by Pritchard et al. (1973), who found no degradation of mirex.

The general history of organochlorine compounds (PCBs, DDT, etc.) entering aquatic ecosystems is well known; they sorb from water to organic matter in sediments, resist microbial degradation, are rapidly accumulated and retained by animals, and move throughout food webs (Woodwell 1967; Hamelink et al. 1971; Metcalf 1977). Our study indicated that Kepone and mirex also resisted microbial degradation, accumulated in fish from food and water, and resisted metabolism in freshwater fish.

In our studies with bluegills, the accumulation potential (as indicated by the accumulation factors) was similar for Kepone and mirex when exposure through water and food was concurrent. However, the retention of mirex introduced to fish only in food was about 3 times greater than that of Kepone. Also,

mirex tended to persist in fish tissue whereas Kepone did not. Presumably, bluegills continuously exposed to mirex would accumulate the compound until death, without reaching an equilibrium. This difference is probably related to the chemical structures of the compounds. Kepone is less lipophilic than mirex and contains a potentially reactive carbonyl group whereas mirex has a strong affinity for fatty tissues and lacks reactive groups that are more easily eliminated (Baetcke et al. 1972). The high accumulation and retention of mirex in fish tissues could be potentially dangerous to higher trophic level consumers. Although the use of these two chemicals has been significantly curtailed, their continued presence in aquatic ecosystems remains a threat to important fishery resources.

Acknowledgments

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Introgressive Hybridization in Populations of Paiute Cutthroat Trout (*Salmo clarki seleniris*)

CRAIG A. BUSACK AND G. A. E. GALL

Department of Animal Science, University of California, Davis, CA 95616, USA

BUSACK, C. A., AND G. A. E. GALL. 1981. Introgressive hybridization in populations of Paiute cutthroat trout (*Salmo clarki seleniris*). Can. J. Fish. Aquat. Sci. 38: 939–951.

Two populations of Paiute cutthroat trout (*Salmo clarki seleniris*) were compared meristically and electrophoretically with Lahontan cutthroat (*S. c. henshawi*) and rainbow trout (*S. gairdneri*) to elucidate population structure and verify the occurrence of introgressive hybridization. In Silver King Creek, both meristic and electrophoretic evidence indicated two populations were present, one appearing to be pure Paiute cutthroat, the other Paiute cutthroat introgressed with rainbow trout. Lahontan cutthroat introgression was a possibility in Silver King Creek but could not be evaluated because of the strong meristic and electrophoretic similarity of Paiute and Lahontan cutthroat. The other Paiute population, Cottonwood Creek, meristically appeared to be pure Paiute cutthroat but electrophoretic data indicated it was introgressed with rainbow trout. The existence of the two Silver King Creek populations indicated introgression was incomplete in that stream; introgression appeared to be complete in Cottonwood Creek. The meristic similarity of Cottonwood Creek trout to pure Paiute cutthroat was probably a result of strong selection by management agencies for a Paiute cutthroat phenotype. Electrophoresis was more discriminating than meristic analysis in this study in detecting introgression. Electrophoresis also allowed more detailed analysis of population structure than meristics because of the difference in complexity of the genetic systems analyzed by the two techniques. However, the application of both techniques contributed greatly to our understanding of introgression in the Paiute cutthroat and demonstrated the complementarity of the two approaches.

Key words: *Salmo clarki*, *Salmo gairdneri*, Paiute cutthroat, Lahontan cutthroat, meristics, electrophoresis, introgression, hybridization, gametic disequilibrium, principal components

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Deux populations de *Salmo clarki seleniris* ont été comparées par les variations méristiques et par électrophorèse à *S. c. henshawi* et à *S. gairdneri*, pour en déterminer la structure et vérifier l'existence d'une hybridation introgressive. Dans le ruisseau Silver King, les variations méristiques et l'électrophorèse ont révélé l'existence de deux populations, l'une étant *S. c. seleniris*, pur, l'autre étant le résultat d'une introgression de cette variété avec la truite arc-en-ciel. Dans le même ruisseau, l'introgression de *S. c. henshawi* était possible, mais n'a pas pu être déterminée à cause de la forte similarité des variations méristiques et des tests d'électrophorèse entre cette espèce et *S. c. seleniris*. L'autre population de *S. c. seleniris*, dans le ruisseau Cottonwood, semblait pure du point de vue des variations méristiques, mais d'après l'électrophorèse, elle avait subi une introgression par la truite arc-en-ciel. L'existence des deux populations dans le ruisseau Silver King signifiait que l'introgression y était incomplète; l'introgression semblait complète dans le ruisseau Cottonwood. La similarité du point de vue des variations méristiques entre la truite du ruisseau Cottonwood et *S. c. seleniris* pur était probablement le résultat d'une forte sélection par les organismes de gestion en faveur du phénotype *seleniris*. L'électrophorèse a permis de mieux déceler l'introgression que l'analyse des variations méristiques; elle a aussi permis une analyse plus détaillé de la structure des populations que les variations méristiques, à cause de la différence de complexité du bagage génétique analysé par ces deux techniques. Toutefois, ces dernières

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aident beaucoup à comprendre le phénomène d'introgression chez *S. c. seleniris* et elles semblent complémentaires l'une de l'autre.

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AT least five closely related trout species of genus *Salmo* inhabit the western half of North America, from the eastern slope of the Rocky Mountains west to the Pacific coast. Natural sympatry of these species is rare, occurring only between rainbow (*S. gairdneri*) and cutthroat trout (*S. clarki*) in coastal drainages (Behnke 1972). Where artificial introductions have created zones of secondary contact between allopatric trout species, hybridization has often resulted or has been assumed to have occurred (Dangel et al. 1973), demonstrating incomplete prezygotic isolating mechanisms in these species. No critical evaluation of postzygotic isolating mechanisms in these fishes is yet available but a great deal of circumstantial evidence suggests that trout hybrids are fertile; introgressive hybridization, the assimilation of genes from one species into the genome of another through hybridization and repeated backcrossing (Anderson 1949), is thought to be a contributing cause of the decline of many western trouts as distinctive forms. In particular, introgressive hybridization with the extensively introduced rainbow trout is an often cited cause of the decline of populations of all other western *Salmo* species (Miller 1950, 1972; Behnke 1965; Schreck and Behnke 1971; Wilmot 1974; Gold and Gall 1975a, b; Behnke and Zarn 1976).

Introgressive hybridization (often simply called introgression) has been a major concern of management agencies attempting to conserve threatened trout forms (see Dangel et al. 1973 for examples). With few exceptions to date, however, research on introgression in trout has emphasized identification of introgressed populations rather than detailed characterization of those populations as has been done in some other fishes (e.g. Greenfield and Greenfield 1972). This paper presents the results of a detailed genetic study of introgressive hybridization in populations of Paiute cutthroat trout (*Salmo clarki seleniris*), a narrowly distributed Lahontan basin form. Polygenic systems were examined by meristic analysis and one- and two-locus systems were examined by starch gel electrophoresis.

The study centered on two populations of Paiute cutthroat with histories of introgression. In both cases, management agencies had attempted to control introgression by removing fish with hybrid characteristics. Our primary interest was the population structure of Silver King Creek. The stream was being managed as if two populations were present, one "hybrid" and one "pure," but other population structures were possible. The other population of interest, Cottonwood Creek, had been returned to a "pure" state by management activities, but it was unclear whether the population was genetically pure or merely phenotypically acceptable to management agencies.

Management History of the Paiute Cutthroat

Except where otherwise noted, the information given below was taken from Ryan and Nicola (1976).

The Paiute cutthroat is native to the Silver King Creek drainage, a portion of the East Carson River drainage of California. Its original range was probably a 6- to 10-km stretch of Silver King Creek immediately below Llewellyn Falls. In 1912, the fish was transplanted by hersdmen to the barren Silver King Creek drainage above the falls. It was also introduced, some time before 1946, into the Corral Valley Creek drainage, which empties into Silver King Creek below Llewellyn Falls. The subspecies subsequently became extinct in its original range, presumably as a result of hybridization with rainbow and Lahontan cutthroat trout (*S. c. henshawi*).

The Paiute cutthroat differs in appearance from the Lahontan cutthroat, the trout native to the rest of the East Carson drainage, only in spotting (Behnke 1965). The body of the Lahontan cutthroat is heavily spotted whereas that of the Paiute is virtually spotless; most of the type specimens collected from Silver King Creek by Snyder (1933) have no body spots but some have as many as nine. This distinctive spotting of the Paiute cutthroat is important to the current study because it has been the basis of the management plan to control introgression.

Rainbow trout were introduced into the Silver King Creek drainage above the falls in 1949 and Lahontan cutthroat were introduced in 1955 or 1956. Heavily spotted trout, presumably the result of hybridization, began appearing in the 1950s. These increased in frequency until only two headwater tributaries of Silver King Creek, Four Mile Canyon and Fly Valley creeks, both above barriers, were free of spotted fish. Waters containing the putative hybrids were chemically treated in 1964 to destroy all fish and were restocked later that year with trout from the hybrid-free creeks.

A 1968 survey found that one-third of the fish in the treated area were heavily spotted. From that time until the inception of the present study in 1976, the management program in the drainage consisted of manually removing spotted fish. The most intensive activity occurred in 1973 and 1974, when the entire area was electrofished and all fish with any body spots were removed. In 1975 only fish with more than five body spots, 28% of those collected, were removed.

Paiute cutthroat from the Corral Valley Creek drainage were introduced into barren waters of the north fork of Cottonwood Creek, Mono County, CA, in 1946. Heavily spotted trout appeared there in 1964, apparently the result of hybridization with rainbow trout introduced from a portion of the stream below a barrier. In 1970, the stream was electrofished and then chemically treated; fish with fewer than five spots, 75% of those collected, were returned to the creek (Wong 1975). The most heavily spotted fish in a large (163 fish) 1973 sample from Cottonwood Creek had only six body spots, no more than some of Snyder's (1933) specimens.

Materials and Methods

Experimental Animals

Seven samples of Paiute and Lahontan cutthroat trout were collected in California in mid-1976 by the California Department of Fish and Game (CDFG) and the U.S. Forest

Service. No rainbow trout were sampled specifically for this study; instead, the electrophoretic data of Busack et al. (1979, 1980) and unpublished meristic data of J. R. Gold and G. A. E. Gall (Department of Animal Science, University of California, Davis, CA) for two domestic rainbow trout strains were used. Designations, descriptions, and sizes (in parentheses) of the nine samples follow.

Paiute cutthroats — FMC — Fourmile Canyon Creek, Alpine County (40). This population was one of those used to restock Silver King Creek after the 1964 treatment. It should represent pure Paiute cutthroat.
NFC — North Fork of Cottonwood Creek, Mono County (19). Fish were sampled where spotted fish had been observed in the past.
SKU — "Unspotted" specimens (having five or fewer body spots) from Silver King Creek above Llewellyn Falls, Alpine County (41).
SKS — "Spotted" specimens (having more than five body spots) from Silver King Creek above Llewellyn Falls (31).

Lahontan cutthroats — INL — Hatchery-reared specimens from eggs taken at Independence Lake, Little Truckee River drainage, Nevada and Sierra counties (43).
MAC — Macklin Creek, Middle Yuba River drainage, Nevada County (30). This population is probably the result of an introduction from the Truckee River drainage (Behnke and Zarn 1976).
MCC — Murray Canyon Creek, East Carson River drainage, Alpine County (29). This population was thought to be an undisturbed remnant population of Lahontan cutthroat.

Rainbow trout — RTH — CDFG's Hot Creek hatchery strain (276 for electrophoresis and 25 for meristics). This is a McCloud River strain with some Mt. Whitney strain ancestry (Busack and Gall 1980).
RTW — CDFG's Mt. Whitney hatchery strain (245 for electrophoresis and 24 for meristics). This strain which was introduced into Silver King Creek in 1949 (Ryan and Nicola 1976) is derived from a variety of native California rainbow trout strains and has some Lahontan cutthroat and Kamloops rainbow trout (*S. g. kamloops*) ancestry (Busack and Gall 1980).

Sample sizes for individual meristic characters and protein systems varied little from the numbers of fish given above, with two exceptions: only 19 INL and 14 MAC fish were available for pyloric ceca counts.

For discussion, the cutthroat samples were divided into two groups: group A consisted of populations in which hybridization was assumed not to have taken place (FMC, INL, MAC, and MCC) whereas group B consisted of populations in which hybridization may have taken place (SKU, SKS, and NFC).

Meristic Analysis

Counts of lateral series scales, vertebrae, pectoral fin rays, pelvic fin rays, branchiostegal rays, gill rakers, and pyloric ceca were made according to Behnke (1965) and Schreck and Behnke (1971). Two nonstandard characters were also counted: proximal dorsal pterygiophores (PDPs) and proximal anal pterygiophores (PAPs). Pterygiophores were counted rather than fin rays because of the ease and accuracy with which they can be counted from radiographs. All *S. clarki* specimens were also examined for the presence of basibranchial teeth.

Sample means, standard errors, and coefficients of variation were calculated for each character in each population. Analysis of variance was carried out for each character to obtain error mean squares for use in further analyses. Means were compared using Student–Newman–Keuls multiple-range tests (Sokal and Rohlf 1969). A multivariate comparison of samples was made by the extraction of principal components from the nine-character correlation matrix. The Euclidean distance between each pair of samples was calculated as the square root of the Mahalanobis distance (Sneath and Sokal 1973). These distances were used to construct an UPGMA dendrogram (Sneath and Sokal 1973).

Electrophoretic Analysis

Horizontal starch gel electrophoresis as described by Busack et al. (1979, 1980) was used to examine 20 loci encoding the following 12 proteins (locus designations given in parentheses): alcohol dehydrogenase (*ADH*), α-glycerophosphate dehydrogenase (*AGPDH-1,2*), creatine kinase (*CK-2*), isocitrate dehydrogenase (*IDH-3,4*), malate dehydrogenase (*MDH-1,2,3,4*), malic enzyme (*ME*), para-albumin (*PALB*), phosphoglucose isomerase (*PGI-1,2,3*), phosphoglucomutase (*PGM*), sorbitol dehydrogenase (*SDH-1,2*), superoxide dismutase (*SOD*), and xanthine dehydrogenase (*XDH*). The SKU and SKS samples were later examined for variation at two peptidase loci using the techniques of Merritt et al. (1978). Glycylleucine was the *PEP-1* substrate and leucylglycylglycine was the *PEP-3* substrate.

Gel interpretation and nomenclature were also as described by Busack et al. (1979, 1980). As in those previous papers, genotypes could not be determined at the individual isocitrate dehydrogenase loci (*IDH-3* and *IDH-4*) nor at two malate dehydrogenase loci (*MDH-3* and *MDH-4*). Thus, *IDH-3,4* and *MDH-3,4* were each treated as systems with two identically polymorphic loci in computing allele frequencies. In addition, para-albumin is now known to be encoded by two loci polymorphic for alleles whose products have identical electrophoretic mobilities (Gall and Bentley 1981). In the present study, however, results were not sufficiently repeatable to score allele dosages confidently, so para-albumin was interpreted as being encoded by a single locus.

Heterozygosities were calculated according to Nei and Roychoudhury (1974). Maximum nonsignificant allele frequency ranges were found using the *G* statistic (Sokal and Rohlf 1969). Standard genetic distances between samples were calculated by the method of Nei (1972) and then used to construct an UPGMA dendrogram.

To facilitate an examination of gametic disequilibrium (often called linkage disequilibrium) in the SKU and SKS samples, loci were made diallelic by pooling the frequencies of minor alleles. The gametic disequilibrium (*D*) for two-locus combinations was estimated using the maximum likelihood method of Hill (1974), with specific procedures suggested by Hedrick et al. (1978), and then expressed as D', the proportion of maximum possible disequilibrium (Lewontin 1964). Disequilibria were tested against a null hypothesis of $D = 0$ using the squared correlation coefficient (r^2) of allele

TABLE. 1. Means ± standard errors and ranges observed in nine meristic characters, percentage of specimens in cutthroat samples possessing basibranchial teeth, and mean coefficients of variation. Means not sharing the same superscript letter are significantly different ($P \leq 0.05$); means without superscripts are significantly different from all others.

	Paiute cutthroats				Lahontan cutthroats			Rainbow trout	
Character	SKU	SKS	NFC	FMC	INL	MAC	MCC	RTH	RTW
Lateral series scales	174.2±1.4^{a} (155−190)	164.6±2.9^{b} (133−201)	168.6±1.7^{a,b} (147−180)	166.2±1.6^{b} (140−184)	157.7±1.4 (137−180)	169.3±2.6^{a,b} (145−200)	174.0±2.0^{b} (153−189)	142.8±0.9 (134−151)	127.0±0.8 (118−137)
Vertebrae	61.5±0.1^{a} (60−63)	62.2±0.2^{b} (61−65)	61.3±0.2^{a} (60−62)	61.7±0.1^{a} (60−63)	61.8±0.1^{a,b} (61−63)	61.2±0.2^{a} (58−62)	62.2±0.1^{b} (60−63)	63.7±0.2 (63−66)	60.4±0.2 (58−62)
Proximal dorsal pterygiophores	12.7±0.1^{a,c} (12−14)	13.4±0.1 (12−16)	12.6±0.1^{a,b,c} (12−13)	12.7±0.1^{a,c} (12−14)	12.2±0.1^{b} (11−13)	12.5±0.1^{a,b} (12−14)	12.6±0.1^{a,b,c} (12−14)	14.0±0.1 (13−15)	13.0±0.1^{c} (12−14)
Proximal anal pterygiophores	12.6±0.1^{a} (11−14)	12.6±0.1^{a} (12−14)	12.6±0.1^{a} (12−14)	12.6±0.1^{a} (12−13)	13.5±0.1 (13−14)	12.8±0.1^{a} (12−14)	12.5±0.1^{a} (12−13)	12.9±0.1^{a} (12−14)	11.4±0.1 (10−13)
Pectoral rays	14.5±0.1^{a} (13−16)	14.0±0.1^{b,c} (13−15)	14.0±0.1^{b,c} (13−15)	14.0±0.1^{b,c} (13−15)	14.4±0.1^{a,c} (12−15)	14.5±0.1^{a} (13−15)	13.8±0.1^{b} (13−15)	15.1±0.1 (14−16)	13.8±0.1^{b} (13−15)
Pelvic rays	9.2±0.1^{a} (9−10)	9.0±0.1^{a} (9−10)	9.0±0.0^{a} (9)	9.1±0.1^{a} (8−10)	9.0±0.0^{a} (9)	9.6±0.1 (9−10)	9.0±0.0^{a} (9−10)	10.2±0.1^{b} (10−11)	10.1±0.1^{b} (9−11)
Branchiostegal rays	11.4±0.1^{a,c} (11−13)	11.5±0.1^{a,c} (11−12)	10.9±0.1^{b} (10−12)	11.6±0.1^{a} (10−12)	10.2±0.1 (9−11)	10.7±0.2^{b} (9−12)	10.9±0.1^{b} (10−12)	11.7±0.1^{a} (11−13)	11.1±0.2^{b,c} (10−13)
Gill rakers	24.5±0.2^{a} (23−27)	22.7±0.4^{b} (19−27)	24.0±0.3^{a,d} (22−27)	24.2±0.2^{a} (22−27)	24.0±0.2^{a,d} (21−27)	23.2±0.2^{b,d} (21−25)	23.7±0.3^{a,d} (21−26)	19.5±0.3^{c} (16−21)	20.2±0.2^{c} (19−21)
Pyloric ceca	59.3±1.5^{a} (40−77)	51.9±1.7^{b,c} (35−67)	53.5±1.1^{b,c,d} (45−61)	57.5±1.0^{a,d} (44−66)	43.7±1.0 (35−51)	50.9±2.1^{b,c} (41−68)	50.7±1.5^{b} (40−75)	57.0±1.3^{a,c,d} (44−70)	54.0±1.2^{b,c,d} (44−63)
Percentage with basibranchial teeth	97.4	46.7	100.0	100.0	100.0	90.0	96.6	—	—
Mean coefficient of variation (%)	5.6	6.6	3.9	4.8	4.8	6.2	5.5	5.3	5.0

frequencies: for a sample of N individuals, $N\hat{r}^2$ has a chi-square distribution with one degree of freedom when $D = 0$ (Hill 1974).

Results

Meristics

Considerable heterogeneity was observed in the group A samples (Table 1). INL was the most distinctive of the four samples, with significantly fewer lateral series scales, branchiostegal rays, and pyloric ceca than the other three samples. As expected, the FMC Paiute cutthroats were meristically very similar to the Lahontan cutthroat samples, differing only in number of branchiostegal rays and pyloric ceca. For both of these characters, the Paiute cutthroats displayed significantly higher means than the Lahontan cutthroats.

The two rainbow trout samples, RTH and RTW, differed significantly in six of the nine characters; in number of pectoral rays, the rainbows exhibited the highest and lowest means of all the samples. The extreme heterogeneity of the rainbow samples limited the number of characters useful in discriminating between rainbow trout and the group A cutthroats. The cutthroats exhibited significantly higher lateral series scale and gill-raker means, but lower pelvic ray means, than the rainbows, all in agreement with the results of Behnke (1965). Basibranchial teeth, treated here as a discrete character state rather than a meristic character, are widely accepted as occurring only in the cutthroat (Behnke 1965; Miller 1950, 1972) and thus discriminate between rainbows and cutthroats.

Two criteria for hybridization were considered in examining the group B cutthroat samples. The first, derived from Hubbs (1955), is that hybrid populations should be intermediate between the parental species in meristic counts. Second, introgressed populations are expected to display high variability relative to the parental forms (Anderson 1949), a consequence of the creation of novel genotypes through genetic recombination.

The unspotted fish from Silver King Creek (SKU) were meristically very similar to the pure Paiute cutthroats (FMC), differing significantly in only two characters, lateral series scales and pectoral rays. In each case, the SKU mean was well within the range of Lahontan cutthroat means. It is unlikely, however, that these differences between SKU and FMC indicate Lahontan cutthroat introgression; for both characters discriminating between Paiute and Lahontan cutthroats, branchiostegal rays and pyloric ceca, the SKU and FMC samples were indistinguishable. The SKU sample also demonstrated a coefficient of variation typical of rainbow trout and group A cutthroats (Table 1).

The spotted fish from Silver King Creek (SKS) exhibited evidence of rainbow trout introgression. SKS differed significantly from the group A cutthroats in number of PDPs and in percentage having basibranchial teeth. The group A samples averaged fewer than 13 PDPs whereas the SKS mean was 13.4, well within the rainbow trout range. Virtually every group A specimen possessed basibranchial teeth but only about half the SKS specimens had them.

When compared with SKU, SKS differed significantly for five additional characters: lateral series scales, vertebrae, pectoral rays, gill rakers, and pyloric ceca. SKS appeared more rainbowlike than SKU in lateral series scales and gill rakers, although the SKS means fell within the range of group A variation. In pyloric ceca, the SKS mean was intermediate between the Lahontan cutthroat and rainbow trout values. This could be taken as an indication of Lahontan cutthroat introgression but the SKS mean was so close to that of NFC, where no Lahontan cutthroat introgression was possible, that it must be regarded as very weak evidence.

The second expected indicator of introgression was high variability relative to the parental forms. SKS specimens exhibited wider ranges of counts (Table 1) than all other samples in lateral series scales, PDPs, gill rakers, and pyloric ceca. Especially conspicuous was the variability in lateral series scales and gill rakers, where the lowest SKS values were well within the rainbow trout range and the highest SKS values equalled or exceeded the highest group A counts. The mean coefficient of variation of SKS for the characters which discriminated between rainbow and cutthroat was about 50% higher than that of all other samples. Considering all characters, the mean coefficient of variation of SKS was again highest but not by nearly so large a margin.

In summary, SKS was meristically distinct from SKU and exhibited evidence of introgression with rainbow trout and possibly with Lahontan cutthroat trout. The evidence fits both the expectations outlined earlier, meristic intermediacy, and high variability.

The final group B sample, NFC, meristically appeared to represent a pure Paiute cutthroat population. NFC was more similar to the pure Paiute cutthroat sample, FMC, than was SKU. NFC differed significantly from FMC only in branchiostegal rays, and here the NFC mean was well within the group A range. None of the NFC specimens was spotted and all had basibranchial teech. NFC also displayed the lowest mean coefficient of variation of all samples, both over all characters and over the four characters discriminating between rainbow and cutthroat trout.

Meristic intermediacy between the putative parental species is a necessary but not a sufficient condition for identification of an introgressed population. Sampling populations consisting of either (1) rainbow and cutthroat trout living in sympatry but not interbreeding or (2) rainbow and cutthroat trout interbreeding but with production of sterile hybrids would result in mean values intermediate between rainbow and cutthroat trout, and high coefficients of variation. To ascertain that introgression has taken place in SKS, backcrossing must be demonstrated. Direct demonstration of backcrossing is impos-

TABLE. 2. Meristic character loadings on principal components I and II. PC I accounted for 29% of the total variance, PC II accounted for 20%.

Character	PC I	PC II
Lateral series scales	0.74	0.44
Vertebrae	−0.28	0.64
PDPs	−0.61	0.36
PAPs	0.24	0.70
Pectoral rays	−0.31	0.60
Pelvic rays	−0.81	−0.08
Branchiostegal rays	−0.34	0.30
Gill rakers	0.81	0.21
Pyloric ceca	−0.12	0.27

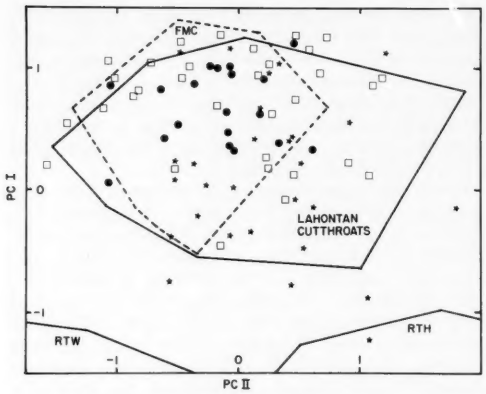

FIG. 1. Principal component scores for Paiute cutthroat, Lahontan cutthroat, and rainbow trout. Individual SKU (open squares), SKS (stars), and NFC specimens (closed circles) are shown; for the other groups, the smallest possible convex polygon bounding the sample space is shown.

sible with meristic analysis but the alternatives (the two hypothetical populations discussed above) can be eliminated by examining the individual specimens in SKS. Both alternatives predict the existence of a breeding population of rainbow trout in Silver King Creek, which presumably would be represented in the SKS sample.

To examine the individual specimens from SKS, principal components were extracted from the nine-character correlation matrix based on all specimens in the study which had counts for all nine characters (219 fish). To facilitate a simple two-dimensional analysis, only the first two components (PC), which accounted for 49% of the variance, were used in the multivariate sample descriptions (Table 2). In PC I, lateral series scales, PDPs, pelvic rays, and gill rakers were weighted most heavily, separating the cutthroats from the rainbows. PC II weighted vertebrae, PAPs, and pectoral rays most heavily; the major effect of this component was to separate the two rainbow trout samples from each other.

The diagram of the bivariate principal component sample spaces (Fig. 1) supports the observations made from the univariate meristic analysis. The sample space of FMC, the pure Paiute cutthroat sample, coincided well with that of the Lahontan cutthroats. SKU was more variable than FMC but the two sample spaces coincided well. NFC had low variability but coincided well with SKU and FMC. Curiously, the introgressed sample SKS was more similar meristically to RTH than to RTW, although RTW was a sample of the strain of rainbow trout which was planted in Silver King Creek. Perhaps RTW was not meristically similar to the Mt. Whitney rainbow of 1949 because of genetic drift or because of the introduction of Kamloops rainbow trout into the Mt. Whitney strain in the mid-1950s (Busack and Gall 1980). The SKS sample space was larger than that of any other sample. Of the 27 SKS specimens examined for all nine characters, only one fell within the rainbow trout space and only six between the Paiute cutthroat space (FMC, NFC, and SKU combined) and the rainbow trout space. No clumping of SKS specimens which could be construed as representing a group of rainbow trout was evident. The conclusion is that the rainbow trout characteristics of SKS are the result of introgressive hybridization; if rainbow trout exist in Silver King Creek, they are rare.

In summary, meristic data indicate the SKU and SKS samples probably represent two populations of trout, one of Paiute cutthroat and the other of Paiute cutthroat introgressed with rainbow trout and possibly Lahontan cutthroat. The alternative hypothesis, that a single introgressed population exists with phenotypic extremes similar to those of pure Paiute cutthroat, is untenable for two reasons. First, it is unlikely a high correlation between characters would exist eight or more generations after hybridization began such that unspotted fish would also have basibranchial teeth, high lateral series scale counts, nine pelvic rays, etc. Second, even if this correlation did exist, there was too high a proportion of unspotted fish in the creek (50% at times) for them to represent the extreme of a normal distribution.

ELECTROPHORESIS

The nine samples were identically monomorphic at five loci: *AGPDH-2*, *MDH-1*, *PGI-2*, *SDH-2*, and *XDH*. The cutthroats from both groups A and B were also identically monomorphic at *CK-2* and *SOD*, loci at which the rainbow trout samples were polymorphic (Busack et al. 1979). At three other loci (*ADH*, *PGI-1*, and *PGI-3*) low level polymorphisms were found but not considered useful in detecting hybridization because the rare allele at each locus was observed in only one sample (MCC in the case of *ADH*; INL in the case of *PGI-1* and *PGI-3*). Allele frequencies at the remaining 10 loci, and heterozygosities calculated over all 20 loci, are presented in Table 3. Maximum nonsignificant allele frequency ranges for selected loci are presented in Fig. 2.

Three of the four group A cutthroat samples, FMC, MAC, and MCC, were virtually identical electrophoretically, all being monomorphic or nearly so for the same alleles at all 10 loci (Fig. 2). This will make electrophoretic detection of Lahontan cutthroat introgression in Silver King Creek impossible. The fourth group A sample, INL, was as distinctive electrophoretically as it was meristically. Besides being substantially more polymorphic than the other samples at *IDH*, *ME*, and *SDH-1*, INL exhibited four alleles not found in any other sample: *AGPDH-1(60)*, *IDH-3,4(115)*, and the variant alleles at *PGI-1* and *PGI-3*.

The group A cutthroats were most distinct from rainbow trout at three loci: *MDH-2*, *ME*, and *SDH-1*. In the case of *MDH-2* and *SDH-1* the two species were nearly monomorphic for alternate alleles. At *ME* the cutthroats exhibited *ME(125)* in high frequency but the rainbows exhibited the *ME(100)* at a frequency of about 0.67 and *ME(85)* at a frequency of about 0.33. *PALB* also helped distinguish between the two species; the cutthroats were nearly monomorphic for *PALB(105)* whereas the rainbows exhibited the *PALB(100)* and *PALB(105)* in approximately equal frequencies. In general, the rainbow trout exhibited more alleles, resulting in higher heterozygosities than the cutthroats. Low within-population heterozygosity of several cutthroat subspecies has been demonstrated by Loudenslager and Gall (1980).

A tacit assumption of the study to this point has been the

TABLE. 3. Allele frequencies at 10 polymorphic structural gene loci, and mean heterozygosities for 20 loci, in Paiute cutthroat, Lahontan cutthroat, and rainbow trout.

		Paiute cutthroats				Lahontan cutthroats			Rainbow trout	
Locus	Allele	SKU	SKS	NFC	FMC	INL	MCC	MAC	RTH	RTW
AGPDH-1	*140*	0	0	0.026	0	0	0	0	0.002	0.009
	100	1.000	1.000	0.974	1.000	0.953	1.000	1.000	0.998	0.991
	60	0	0	0	0	0.047	0	0	0	0
IDH-3,4	*170*	0	0	0	0	0.214	0	0.150	0.001	0.382
	140	1.000	1.000	0.987	1.000	0.488	1.000	0.850	0.699	0.340
	115	0	0	0	0	0.268	0	0	0	0
	100	0	0	0.013	0	0.030	0	0	0.173	0.075
	60	0	0	0	0	0	0	0	0.127	0.203
MDH-2	*130*	0.939	0.433	0.526	0.988	0.900	0.914	0.967	0	0.014
	100	0.061	0.567	0.474	0.012	0.100	0.086	0.033	1.000	0.986
MDH-3,4	*125*	0	0.097	0	0	0	0	0	0	0.006
	100	0.994	0.822	1.000	1.000	1.000	1.000	1.000	0.999	0.750
	85	0.006	0.081	0	0	0	0	0	0.001	0.244
ME	*125*	0.951	0.661	0.842	1.000	0.893	1.000	1.000	0	0
	100	0.049	0.339	0.158	0	0.107	0	0	0.697	0.645
	85	0	0	0	0	0	0	0	0.303	0.355
PALB	*105*	0.962	0.683	0.816	1.000	0.930	1.000	1.000	0.462	0.552
	100	0.038	0.317	0.184	0	0.070	0	0	0.538	0.448
PGM	*100*	1.000	1.000	0.895	1.000	1.000	1.000	0.967	0.684	1.000
	85	0	0	0.105	0	0	0	0.033	0.316	0
SDH-1	*250*	0.988	0.839	0.964	1.000	0.779	1.000	1.000	0	0
	40	0.012	0.161	0.036	0	0.221	0	0	1.000	1.000
Heterozygosity		0.017	0.116	0.071	0.001	0.142	0.010	0.032	0.125	0.181

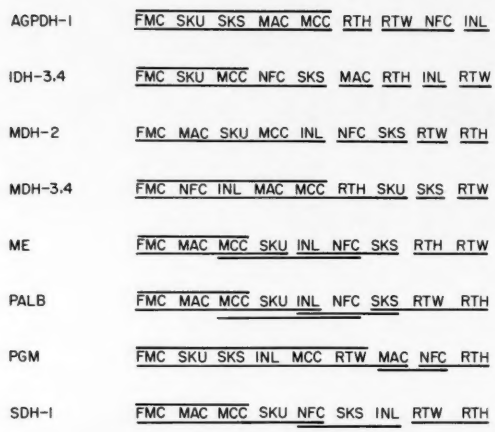
AGPDH-1
FMC SKU SKS MAC MCC RTH RTW NFC INL
IDH-3,4
FMC SKU MCC NFC SKS MAC RTH INL RTW
MDH-2
FMC MAC SKU MCC INL NFC SKS RTW RTH
MDH-3,4
FMC NFC INL MAC MCC RTH SKU SKS RTW
ME
FMC MAC MCC SKU INL NFC SKS RTH RTW
PALB
FMC MAC MCC SKU INL NFC SKS RTW RTH
PGM
FMC SKU SKS INL MCC RTW MAC NFC RTH
SDH-1
FMC MAC MCC SKU NFC SKS INL RTW RTH

FIG. 2. Relationships among Paiute cutthroat, Lahontan cutthroat, and rainbow trout in allele frequencies. Samples not underlined by same line differed significantly in frequency ($P \leq 0.05$); overlined samples were identically monomorphic.

genetic purity of the Lahontan cutthroat samples. Loudenslager and Gall (1980), in their extensive sampling of the Lahontan basin, found many of the same rare cutthroat alleles found in this study. In some cases, however, the occurrence of these alleles correlated well with planting records of rainbow trout (E. J. Loudenslager, Department of Animal Science, Univ. of California, Davis, CA, personal communication). It must be acknowledged, then, that some of the low level polymorphism seen in the group A samples may reflect rainbow trout introgression. This possibility is illustrated by MAC, which is weakly polymorphic at three protein systems. The INL sample, on the other hand, displayed some polymorphisms which can be explained by rainbow trout introgression (*MDH-2*, *ME*, *PALB*, *SDH-1*), but also other polymorphisms which cannot (*AGPDH-1*, *IDH*, *PGI-1*, *PGI-3*). The conclusions yet to be drawn regarding the purity of the group B samples will not be affected if the group A samples do in fact exhibit low level introgression.

In the meristic assessment of the group B samples, two possible criteria for detection of introgression were used, intermediacy and high variability relative to the parental species. The same criteria are appropriate for electrophoretic analysis, but with qualifications, because in this case the two criteria are not independent. The presence of both rainbow and cutthroat alleles would indicate intermediacy and would normally result in higher heterozygosity, the common measure of genetic variability. However, in the present study, the large discrepancy in heterozygosity between rainbow and cutthroat trout (Table 3) made it unlikely that introgressed populations would be more variable than rainbows. Consequently, high variability in a cutthroat population would be taken as evidence of introgression if rainbow trout alleles also occurred in the population.

SKU was electrophoretically very similar to FMC. It did, however, exhibit low level polymorphisms at five systems (*MDH-2*, *MDH-3,4*, *ME*, *PALB*, *SDH-1*). One allele found in SKU (*MDH-3,4(85)*) was found in the rainbow trout samples but not in the group A samples or in any of the Lahontan cutthroats sampled by Loudenslager and Gall (1980. Thus, SKU exhibited electrophoretic variation consistent with a hypothesis of low level rainbow trout introgression.

SKS was electrophoretically distinct from SKU and displayed genetic variation consistent with the idea of rainbow trout introgression. It differed significantly from the group A samples at *MDH-2*, *ME*, and *PALB*. At *MDH-2* the common rainbow and cutthroat alleles occurred in approximately equal frequencies in SKS. *PALB(100)*, also common in rainbow trout, occurred in much higher frequency than in the group A samples. *ME(125)* was found in lower and *ME(100)* in higher frequency than in the group A fish. Another difference between SKS and the group A samples, although not a statistically significant one, was the presence in SKS of two *MDH-3,4* rainbow trout alleles which were absent from the group A samples. SKS also differed from SKU in the frequency of *SDH-I(40)*, the common rainbow allele.

Additional differences between SKU and SKS were provided by E. J. Loudenslager (Department of Animal Science, Univ. of California, Davis, CA, unpublished data), who compared the two samples at two diallelic peptidase loci which readily distinguish rainbow from cutthroat trout (E. J. Loudenslager and G. A. E. Gall, Department of Animal Science, Univ. of California, Davis, CA, unpublished data). At both *PEP-1* and *PEP-3*, SKS exhibited the rainbow trout alleles, *PEP-1(100)* and *PEP-3(100)*, in significantly higher frequencies (0.267 and 0.350) than did SKU (0.012 and 0.038).

NFC, which appeared meristically to be pure Paiute cutthroat, exhibited electrophoretic variation consistent with the hypothesis of rainbow trout introgression. The most compelling evidence of this was found at *MDH-2* where, like SKS, NFC displayed the common rainbow and cutthroat alleles in intermediate frequencies. NFC was also the only cutthroat sample to display *AGPDH-1(40)*, an allele found in low frequency in the rainbow trout samples. Finally, at *ME*, *PALB*, and *PGM*, NFC had common rainbow trout alleles in higher frequencies than in FMC and SKU, though not in significantly higher frequencies than all the group A samples. Corroborating evidence that NFC represented an introgressed population was later obtained from a sampling of an isolated upstream section of Cottonwood Creek where no spotted fish had ever been found. The fish in the new sample displayed none of the polymorphisms found in the NFC fish (G. A. E. Gall, Department of Animal Science, Univ. of California, Davis, CA, unpublished data).

A multivariate approach to the electrophoretic data was undertaken to verify that introgressive hybridization had

TABLE. 4. Frequency distribution of rainbow trout allele scores in SKU, SKS, and NFC. Maximum possible scores are 12 in SKU and SKS, 10 in NFC.

	Allele scores									
Sample	0	1	2	3	4	5	6	7	8	9
SKU	35	1	2	1	1	—	—	—	—	—
SKS	3	2	6	3	4	5	1	—	4	1
NFC	4	6	6	2	—	—	1	—	—	—

TABLE. 5. Estimates of D' and r^2 (in parentheses) in the SKU and SKS samples for 15 two-locus combinations. Asterisks indicate significance for tests against a null hypothesis of no disequilibrium (Hill 1974) (*$P \leq 0.05$; ***$P \leq 0.005$).

	PEP-3		*MDH-2*		*MDH-3*		*ME*		*SDH-1*	
	SKU	SKS	SKU	SKS	SKU	SKS	SKU	SKS	SKU	SKS
PEP-1	1.000*** (0.329)	0.186 (0.023)	1.000*** (0.494)	0.311 (0.082)	0.994*** (0.988)	0.256 (0.041)	1.000 (0.001)	0.488* (0.172)	1.000 (0.000)	0.118 (0.007)
PEP-3			0.480* (0.152)	0.400* (0.137)	1.000*** (0.329)	0.439* (0.179)	0.292 (0.063)	0.372 (0.128)	1.000 (0.000)	0.154 (0.008)
MDH-2					1.000*** (0.494)	0.541*** (0.255)	0.474* (0.110)	0.227 (0.044)	1.000 (0.000)	0.192 (0.014)
MDH-3							1.000 (0.001)	0.328 (0.087)	1.000 (0.000)	0.380 (0.047)
ME									1.000*** (0.247)	0.431 (0.018)

occurred. Each SKU and SKS specimen was scored for the number of rainbow trout alleles present at *PEP-1*, *PEP-2*, *MDH-2*, *MDH-3*, *ME*, and *SDH-1*. All low level polymorphism was assumed to be derived from rainbow trout. It was also assumed that all *MDH-3,4* variation occurred at *MDH-3*, probably a good assumption as *MDH-4* variation has been found in only a very few rainbow trout (Busack et al. 1979) and no cutthroat trout. By this scoring scheme, pure cutthroats would have scores of 0; pure rainbow trout would have scores of 10–12. A similar scoring scheme was followed for NFC but using only five loci (*AGPDH-1*, *MDH-2*, *ME*, *PGM*, and *SDH-1*), so scores ranged from 0 to 10. Resulting frequency distributions of allele scores demonstrate that no rainbow trout were found in any of the three samples (Table 4). In SKU, 35 of 40 specimens were pure cutthroat, compared with 4 of 29 in SKS and 4 of 19 in NFC.

Because electrophoretic variants at different loci assort independently when loci are unlinked, as all of these can be assumed to be (May et al. 1979), each fish's pattern of variation over all loci reveals something of its ancestry. An F_1 hybrid would be expected to have one rainbow trout allele at each locus. A fish exhibiting no rainbow trout alleles at one locus, but two at another, is most parsimoniously explained as an F_2 or backcross individual. Specimens demonstrating this type of variation are evidence of the fertility of hybrids and, thus, proof of introgression. None of the specimens in SKU, SKS, or NFC could be identified as F_1 hybrids, even assuming the low level cutthroat polymorphisms to be natural. All fish in the three samples appeared either to be pure cutthroat or the product of rainbow trout introgression.

For SKU to be merely the unspotted extreme of a single randomly mating population, alleles at five or six loci would have to be nearly perfectly correlated with spotting. It is much more likely that the electrophoretic evidence, like the meristic, demonstrates the existence of two populations in Silver King Creek, an essentially pure Paiute cutthroat and a population introgressed with rainbow trout.

The fish in the SKU sample which exhibit introgression are possibly the product of sampling error. Assuming a normal distribution of body spotting in the introgressed population, some fish would have very few spots and could have been included in the SKU sample. As the two populations differ in allele frequency at several loci, mixing them in the SKU sample should cause gametic disequilibrium (Cavalli-Sforza and Bodmer 1971). Conversely, if pure Paiute cutthroat have up to nine body spots, the five-or-fewer spotting criterion may have assigned a few fish from the pure population to SKS. This should also cause gametic disequilibrium.

All two-locus combinations of *PEP-1*, *PEP-3*, *MDH-2*, *MDH-3*, *ME*, and *SDH-1* were examined for gametic disequilibrium in SKU and SKS. Eight of the 15 SKU combinations showed significant disequilibrium (Table 5), with D' ranging from 0.47 to 1.00. Theoretically, these disequilibria could be caused by selection but gametic disequilibria between allozyme loci in natural populations are seldom observed, even in *Drosophila* (Charlesworth et al. 1979), which have as few as three linkage groups. Thus, these large values of disequilibrium in SKU are probably the product of population mixing. Inspection of SKU genotypes revealed that a large amount of variation, including all *MDH-3* and *PEP-1*

TABLE. 6. Euclidean distances, based on nine meristic characters (above diagonal), and genetic distances, based on 20 loci (below diagonal), for all populations.

	FMC	SKU	SKS	NFC	INL	MAC	MCC	RTH	RTW
FMC	—	1.05	1.88	1.82	4.31	2.45	2.12	6.46	6.56
SKU	0.000	—	2.21	1.61	4.05	2.77	2.05	6.48	6.83
SKS	0.032	0.026	—	2.41	4.18	3.30	2.11	5.55	6.46
NFC	0.015	0.012	0.008	—	2.95	2.45	1.76	6.91	6.84
INL	0.030	0.029	0.054	0.041	—	3.10	3.67	7.06	7.56
MAC	0.002	0.003	0.034	0.017	0.021	—	2.83	5.67	6.05
MCC	0.000	0.000	0.027	0.011	0.030	0.003	—	6.87	7.33
RTH	0.273	0.258	0.165	0.195	0.252	0.272	0.260	—	6.00
RTW	0.234	0.220	0.123	0.165	0.175	0.218	0.222	0.083	—

variation, was found in a single fish. Deleting this fish from the sample would reduce the number of significant disequilibria to three. These could be removed by deleting two more fish. Assuming, then, that all SKU disequilibrium was caused by mixing, the five-or-fewer spotting criterion allowed three fish from the introgressed population into the SKU sample of 41 fish.

Among the SKS trout, 5 of 15 comparisons were significant, with D' ranging from 0.38 to 0.49. Examination of the three preserved specimens which exhibited no rainbow trout alleles revealed they had 4, 10, and 20 body spots; deleting the first two fish from the SKS sample would still leave two significant disequilibria. The persistence of the disequilibria may indicate interbreeding between the pure population and the introgressed population (Nei and Li 1973).

Comparison of Meristic and Electrophoretic Results

Euclidean and genetic distances (Table 6) for each pair of samples were highly correlated ($0.90 \leq$ Spearman's rank correlation coefficient $(r_s) \leq 0.97$, with ~95% confidence). This was due, in part, to the inclusion of rainbow trout, which were quite distinctive both electrophoretically and meristically from the cutthroats. Among cutthroat samples alone the correlation was still significant ($0.25 \leq r_s \leq 0.82$, with ~95% confidence).

Phenetic relationships based on the distance calculations are presented in Fig. 3 and 4 as UPGMA dendrograms. Meristically, there was no clear pattern of relationship among the cutthroat populations except FMC and SKU were similar to each other and INL was distinct from the other cutthroats. In contrast, genetic distance estimates suggested that the SKU and FMC Paiutes were virtually identical with the MCC and MAC Lahontan cutthroats. Second, SKS and NFC were more closely related to each other than to the other cutthroats. It was the presence of rainbow trout alleles in SKS and NFC that resulted in this clustering of the introgressed populations.

There was no correlation between meristic measures (coefficients of variation) and genetic measures (heterozygosities) of intrasample variability. A large difference in heterozygosity was seen between rainbow and cutthroat trout (excluding INL); within the cutthroats, samples of introgressed populations were more heterozygous than nonintrogressed populations. Meristically no such trend existed. One of the introgressed samples, NFC, had the lowest mean coefficient of variation of all samples while the other, SKS, had the highest.

A bivariate principal component scattergram of SKS and NFC specimens, with their rainbow trout allele scores added, is presented in Fig. 5. SKS specimens with extremely negative PC I and high positive PC II coordinates also tended to have high rainbow trout alleles scores. Thus, within this sample, meristic outliers tended to be electrophoretic outliers. Also, the specimens meristically nearest to the rainbow trout sample spaces had high allele scores. In NFC no correlation between principal component coordinates and alleles scores was evident.

Discussion and Conclusions

Silver King Creek contains two trout populations: one appears both meristically and electrophoretically to be pure Paiute cutthroat, the other appears by both analyses to be the product of rainbow trout introgression. In contrast, Cottonwood Creek contains fish which meristically appear to be Paiute cutthroats but electrophoretically show clear evidence of rainbow trout introgression. The high meristic uniformity (Table 1, Fig. 1), unimodal distribution of rainbow trout alleles (Table 4), and lack of correlation between principal component and allele scores (Fig. 5) in the NFC sample all indicate that only one population is present.

The difference in population structure in the two creeks may be explained by introducing a simple introgression model. Consider a stream inhabited by species A into which species B is introduced. Let x_0 denote the frequency of species A in the breeding population at the first breeding season in which both species participate. Assuming constant population size, discrete generations, random mating, and equal fitness of all fish, the frequency of purebred species A fish t generations after mixing (x_t) will be $(x_0^2)^t$. Under this model, the frequency of species A drops to a negligible level very rapidly unless x_0 is close to 1 (Fig. 6). Introgression may be considered complete when purebred species A fish no longer exist.

A conservative estimate of the population size of Silver King Creek is 2000 fish (J. H. Ryan, California Department of Fish and Game, personal communication); Wong (1975) estimated the Cottonwood population at 500 fish. This disparity in population sizes makes the theoretical impact per rainbow trout transplanted much greater in Cottonwood Creek than in Silver King Creek. Not enough is known to estimate x_0 for either population, but it is known that in Silver King Creek the rainbow trout were the survivors of a single introduction of 5000 hatchery fingerlings (Ryan and Nicola 1976).

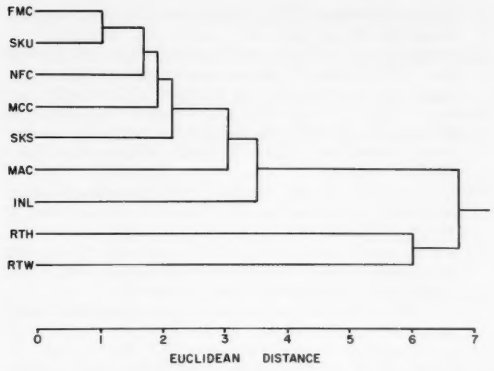

FIG. 3. Euclidean distance dendrogram based on means of nine meristic characters. The cophenetic correlation coefficient is 0.98.

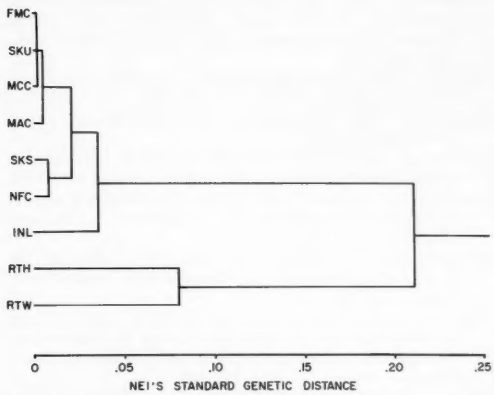

FIG. 4. Genetic distance dendrogram based on allele frequencies at 20 structural gene loci. The cophenetic correlation coefficient is 0.96.

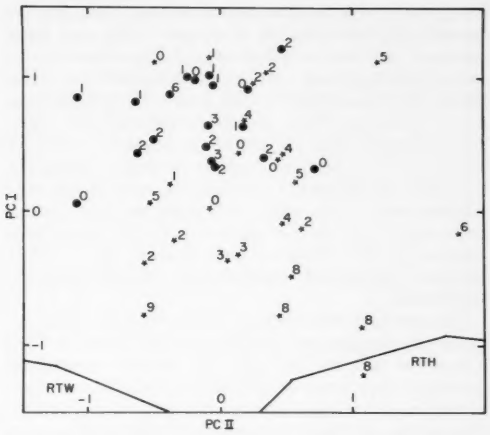

FIG. 5. Principal component scores and rainbow trout allele scores of SKS (stars) and NFC specimens (closed circles).

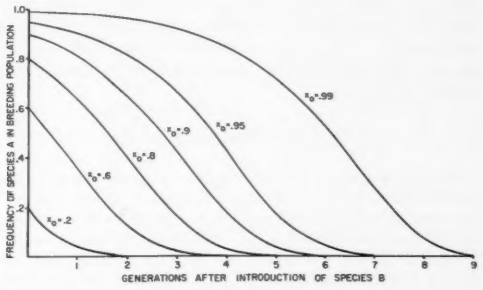

FIG. 6. Continuous-time representation of decline in frequency of purebred species A after species B is introduced, assuming constant population size, discrete generations, random mating, and equal fitness of all individuals. Original frequency of species A is denoted by x_0.

Trout fingerlings typically have a low survival rate, so x_0 in Silver King Creek may have been quite large. In contrast, the rainbow trout in Cottonwood Creek came from an established population downstream from the Paiutes. These transplanted fish would be expected to have a high survival rate and several transplantations may have occurred. These factors could easily have made the effective x_0 much lower in Cottonwood Creek than in Silver King Creek. In light of the model, the difference in population structure between the two creeks probably does not reflect a difference in the outcome of introgression but, rather, different stages in the process; introgression is not yet complete in Silver King Creek but is complete in Cottonwood Creek.

Management agencies have doubtless retarded the introgression process in Silver King Creek by removing large numbers of fish from the introgressed population but there is evidence that introgression may have also been retarded by assortative mating. When the SKU and SKS samples were collected, spotted fish were much more common in fast water than in slow water. Probably 75% of the SKS fish were collected in fast water. Interbreeding between the pure and introgressed populations in Silver King Creek may thus be largely limited to the margins of fast water areas. This habittat preference may simply be a case of fish with rainbow trout ancestry preferring "rainbow trout-like" habitat (Mc Afee 1966) or it may be a case of introgressed fish better exploiting a new habitat than nonintrogressed fish. Anderson (1949) speculated that introgression commonly leads to the occupation of new habitats and Lewontin and Birch (1966) demonstrated experimentally that it is possible.

The Cottonwood Creek population provided the major discrepancy of the study; NFC fish electrophoretically showed clear evidence of rainbow trout introgression yet meristically were indistinguishable from pure Paiute cutthroat. This situation most likely did not arise naturally. Spotted fish were common in Cottonwood Creek before the 1970 chemical treatment and rainbow trout still live downstream from the

Paiute cutthroat population, below a barrier. Thus, it is improbable that the situation of meristics disguising introgression is caused by natural selection. It was probably caused instead by management activities. As polygenic traits tend to remain highly correlated for some time in introgressed populations (Anderson 1949), and as perhaps only three generations elapsed between the time of rainbow trout introduction and the 1970 chemical treatment, the removal of spotted fish during the treatment may have effectively selected against all rainbow trout meristic traits. This strong artificial selection could also be the cause of the low coefficients of variation observed in NFC. Rainbow trout allozyme alleles, which segregate independently, could not have been removed nearly as efficiently.

Electrophoretic and meristic evidence led to the same conclusion regarding the population structure of the trout in Silver King Creek whereas electrophoresis detected introgression not detected by meristic analysis in Cottonwood Creek. Electrophoresis also allowed more detailed analysis of population structure than did meristics because it examines much simpler genetic systems than do morphological methods such as meristics. The conclusion should not be made, however, that electrophoresis is superior to meristics in studying introgression in trout. Both techniques yield useful, often complementary information; we learned significantly more about introgression in the Paiute cutthroat by using both electrophoresis and meristics than we could have by using either method alone.

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Determination of Mineral Fiber Concentrations in Fish Tissues

ALLAN R. BATTERMAN AND PHILIP M. COOK

U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, MN 55804, USA

BATTERMAN, A. R., AND P. M. COOK. 1981. Determination of mineral fiber concentrations in fish tissues. Can. J. Fish. Aquat. Sci. 38: 952–959.

Submicroscopic inorganic particle concentrations in tissue have not been quantitatively determined in the past because of a lack of sample preparation techniques capable of achieving the sensitivity required. The determination of whether mineral fibres in water are accumulated in aquatic organisms requires transmission electron microscope examination of bulk tissue residues rather than thin sections. The sample preparation method used for this investigation involved removal of water and organic matter by freeze-drying and low temperature ashing. Lake trout with a lifetime exposure to Lake Superior water containing amphibole fibers contained similar amphibole fibers particularly in the kidney and with low concentrations in muscle tissue. Lake trout from two locations with widely different water fiber concentrations had corresponding differences in tissue fiber concentrations. Analysis of other fish raised under laboratory conditions suggests that ingestion is the primary route for fiber accumulation.

Key words: electron microscopy, fiber accumulation, asbestos, lake trout, brook trout, channel catfish, Arctic char

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Le manque d'une technique de préparation des échantillons permettant d'atteindre la sensibilité requise est la raison pour laquelle les concentrations de particules inorganiques submicroscopiques dans les tissus n'ont pu être mesurées quantitativement dans le passé. Afin de déterminer si des fibres minérales présentes dans l'eau s'accumulent dans les organismes aquatiques, il faut examiner au microscope électronique des tissus en vrac plutôt que de minces coupes. Dans l'étude décrite ci-dessous, la préparation des échantillons comporte l'enlèvement de l'eau et de la matière organique par cryodessication et incinération à basse température. Des touladis ayant été exposés toute leur vie à l'eau du lac Supérieur contenant des fibres d'amphibole ont également de ces fibres, particulièrement dans le rein et, à de faibles concentrations, dans le tissu musculaire. Des touladis provenant de deux endroits dont la concentration des fibres dans l'eau était très différentes montrèrent des différences correspondantes de concentration des fibres dans les tissus. L'analyse d'autres poissons élevés dans des conditions de laboratoire donne à penser que l'ingestion est la principale voie d'accumulation des fibres.

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RECENT concern over the human carcinogenicity of asbestos and analogous mineral fibers when ingested (Lee 1974) has spurred interest in the development of methods for the quantitative determination of their presence in tissues. The discovery of mineral fibers in human urine has demonstrated that some fibers can pass through the human gastrointestinal tract following ingestion of water contaminated with amphibole[1] fibers (Cook and Olson 1979). The same type of fibers were

[1]Amphibole minerals are members of a class of hydrated silicates having a double-chain crystal structure. These minerals can crystallize during rock formation in an asbestiform habit (bundles of individual fibers). Amphibole minerals used commercially as asbestos (when minable as the asbestiform variety) are amosite (fibrous grunerite), crocidolite, anthophyllite, tremolite, and actinolite. Most amphibole particles in western Lake Superior water fall in the cummingtonite–grunerite series and appear to range from nonasbestiform to asbestiform in origin. A fiber in a water sample is defined as any particle with a length-to-width ratio equal to or exceeding 3:1. Some nonasbestiform amphibole mineral crystals, when crushed, form cleavage fragments that are microscopically very similar to, or identical with, small fibers, which result from the crushing of amphibole asbestos, in morphology, crystal structure, and chemistry. About 95% of the asbestos used in North America is chrysotile, a fibrous serpentine mineral with a distinct tubular microstructure.

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TABLE 1. Fish samples analyzed.

Species	Location	Age (years)	Collection date (mo/yr)	No. of fish	Estimated avg. water fiber concn. (fibers/L)		Food source
					Amphibole $\times 10^6$	Chrysotile $\times 10^6$	
Salvelinus namaycush	Split Rock, Minnesota	3–4	May 1978	2	100	< 1	Natural diet
S. namaycush	Huron Bay, Michigan	4–5	March 1980	1	1	Unknown	Natural diet
S. alpinus	Deception Bay, Hudson Bay, Quebec	7–12	Sept. 1978	2	Unknown	0–500	Natural diet
S. fontinalis	ERL-D, Duluth, Minnesota	2–3	Feb. 1980	1	50	< 1	Lab diet (Glencoe® Pellets)
Ictalurus lacustris	ERL-D, Duluth, Minnesota	5–6	May 1976	2	50	< 1	Lab diet (Glencoe® Pellets)

recently reported to be present in human liver, lung, and jejunum tissues as a consequence of ingesting Lake Superior water containing amphibole fibers (Carter and Taylor 1980). No studies of the accumulation of fibers by aquatic organisms have been reported with the exception of the optical microscope observation of particles in mussels (*Mytilus edulis*) exposed to asbestos tailings (Halsband 1974). Because mineral fibers contaminate some natural waters and fish are thus exposed to these fibers, we have investigated the possible accumulation of these fibers in fish tissue. Optical microscope examination of tissue preparations for mineral fibers is not practical because most of these particles have widths less than the resolving power of the optical microscope (Berkeley et al. 1965). Electron microscopy of standard histological preparations of ashed thin sections is not feasible because the approximately 70-nm-thick tissue sections would require astronomical numbers of fish in tissue to see one fiber in an entire section (Cook 1979). Since detection of concentrations of only a few hundred fibers or less per gram of tissue is required, a method is necessary which separates the fibers from larger pieces of tissue without altering them. This method must be able to eliminate all organic matter without changing the morphological, chemical, or physical characteristics of fibers.

To determine if freshwater fish used as human food can accumulate mineral fibers in their tissue, either by contact with the particles in contaminated waters or by ingestion of contaminated organisms of the food chain, several species of fish with different exposure histories were selected for analysis.

Methods

Exposure concentrations for fish collected from natural waters (Table 1) are approximations based on the assumption that the fish spent a significant portion of the year prior to capture in the collection area rather than distant areas with radically different fiber concentrations. All fish except the brook trout were frozen for storage and/or shipment prior to thawing and removal of tissue samples for analysis. Brook trout were collected from laboratory stock at the time of tissue preparation. Arctic char samples were obtained from frozen tail sections received after other portions of the fish were analyzed for toxic chemicals (I. Boulva and A. LeBeau, Department of Fisheries and Oceans, C.P. 15500, Quebec, Que. G1K 7Y7, 1979, personal cummunication).

The fish were dissected in a filtered air laminar flow hood in a clean room with a positive-pressure particle-free air supply. Dissecting instruments were cleaned with particle-free water. One set of instruments was used to remove the skin, another set to remove a block of tissue, and a third set to remove a subsection of tissue for actual analysis. The tissue sample was placed in a 25-mL Pyrex sample tube, weighed, frozen in liquid nitrogen, freeze-dried for ~ 5 h, and placed in a low temperature asher (LTA) at 150 W and 1.5 mL O_2/min for at least 16 h. The ash was suspended in ~ 15 mL of water acidified with 6 drops of 6 M HCl. Blank samples were prepared by processing an empty tube with the tissue sample tubes in each chamber of the LTA. The ash suspension was placed in an ultrasonic bath for 2 min, mixed on a vortex stirrer for 1 min, and filtered through a 25-mm 0.1-μm Nuclepore membrane filter. The filter was placed in a plastic Petri dish and dried for ~ 30 min at 30°C. One-half of the filter was carbon-coated in a vacuum evaporator. Small pieces (2 mm^2) were cut out of the carbon-coated filter and placed carbon side down on Formvar-film-coated electron microscope finder grids. The grids were placed on a piece of coarse metal screen on top of several layers of filter paper in a Petri dish. A small drop (10 μL) of 0.1-μm filtered chloroform was placed on each filter section and then the layers of filter paper were immediately saturated with filtered chloroform. The Petri dish was covered, and the chloroform allowed to dissolve all of the filter piece and Formvar film for ~ 12 h. The grid which resulted was covered with a thin carbon film in which sample particles were imbedded (Fig. 1). This technique prevents the loss of particles during transfer of the sample from the filter to the grid, preserves the distribution of particles originally in the sample, and leaves a replica of the filter (0.1-μm holes) which can be used to check on folds or breaks in the carbon film which would create areas of uneven particle distribution.

Randomly chosen grid openings with areas of ~ 9500 μm^2 were systematically scanned at a magnification of 10 000× or higher on a JEOL 100C electron microscope in the trans-

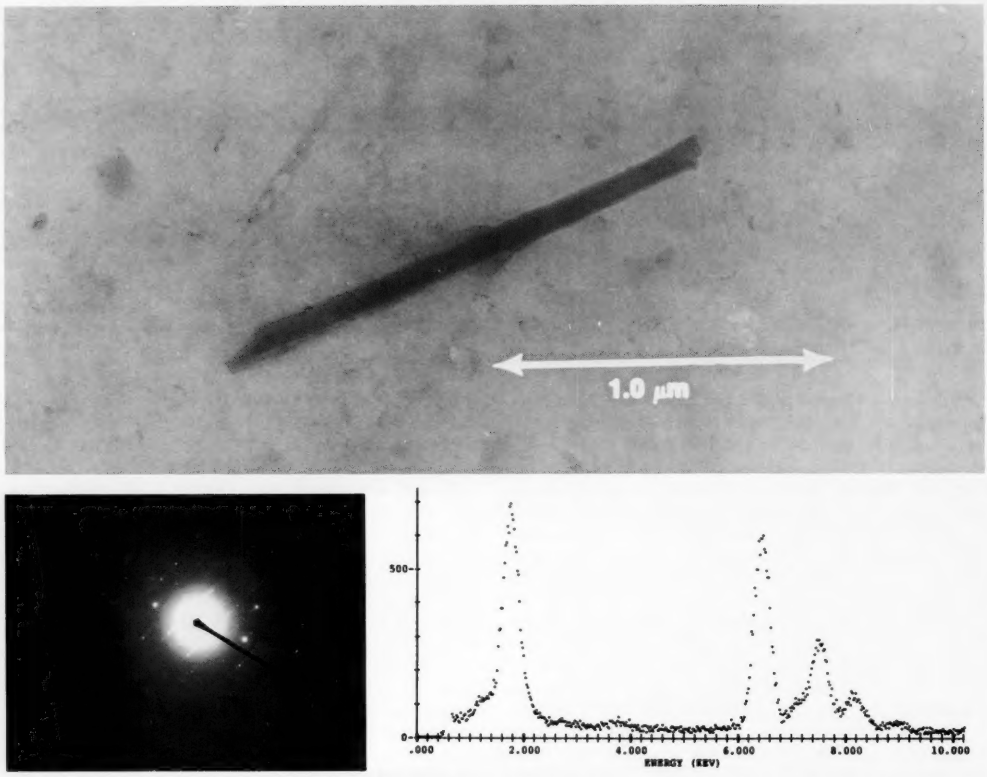

FIG. 1. Electron micrograph of a fiber, 1.6 μm long, identified in a lake trout kidney preparation. The selected area electron diffraction pattern (*lower* left insert) established the mineral group as amphibole. The energy-dispersive X-ray spectrum (*lower* right insert) with major silicon and iron peaks established the mineral as grunerite.

mission mode. Grid openings with broken carbon film or other disruption of the sample were rejected. Each particle with an aspect (length to width) ratio ≥3 was identified by camparison of its morphology, selected area electron diffraction (SAED) pattern, and energy dispersive X-ray (EDX) spectrum with those obtained for standard mineral samples. Amphibole (Fig. 1) and chrysotile fibers produce characteristic SAED patterns and EDX spectra when oriented properly in the electron beam. Fiber size, orientation, or interfering particles cause difficulties with positive identification of fiber mineralogy. Fibers which possess amphibole or chrysotile morphology but SAED patterns and/or EDX spectra not clearly diagnostic for amphiboles, chrysotile, or some other mineral were classified as "ambiguous." Each sample was examined until 100 particles of interest were counted or at least 10 grid openings were scanned.

Fiber concentrations were calculated from the formula:

$$\text{No. of fibers/mg} = \frac{\text{no. of fibers} \times \text{filtration area } (\mu m^2)}{\text{area examined } (\mu m^2) \times \text{weight of sample (mg)}}.$$

Fiber size distributions were analyzed and compared by computer program. Elemental ratios calculated from integrated EDX peak intensities were plotted on ternary diagrams to facilitate the comparison of mineral fiber elemental compositions for different samples.

The tissue preparation technique descibed above was chosen after attempting several other techniques involving chemical digestion. The principle objective was to optimize the sensitivity of the analysis by increasing the amount of tissue processed per unit area of electron microscope grid examined. Digestion chemicals included Soluene®, KOH, H_2O_2, and a mixture of KOH−H_2O_2. Although these reagents all effectively digested muscle, kidney, and liver tissues, the digests when filtered through a Nuclepore filter (either 0.1-μm or 0.4-μm pore diameter) left too much residue to allow direct preparation of electron microscope grids. Therefore all chemical digestion residues on filters had to be low temperature ashed and refiltered prior to grid preparation. The resulting grids were suitable for electron microscope analysis and generally contained a slightly greater sample density than grids prepared by LTA without chemical digestion. The

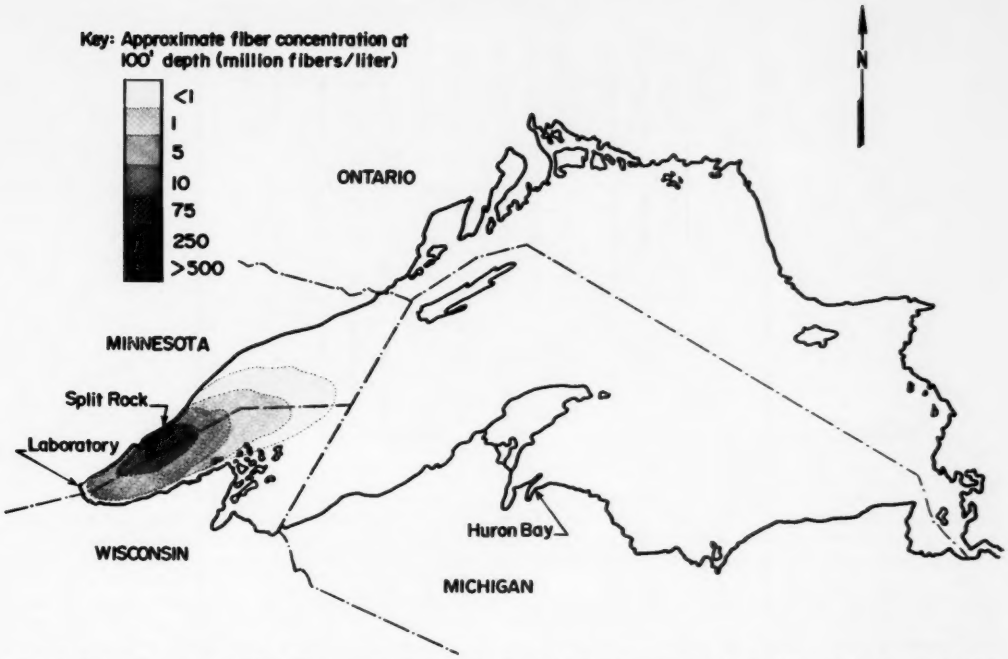

FIG. 2. Estimated average amphibole fiber concentrations in subsurface Lake Superior waters at a depth of 30 m during the period of fish exposure influencing this study. The approximated distribution pattern is based on western Lake Superior water circulation, available measurements of fiber concentrations, and X-ray diffraction measurements of the amphibole mineral content of suspended solids and bottom sediments (Cook et al. 1976).

chemical digestion techniques were not chosen for this study, however, because membrane filters when ashed with the sample were found to add significant numbers of chrysotile fibers. Amphibole asbestos (crocidolite) fibers have also been found in one batch of membrane filters. Since the chemical digestion techniques also involved considerably more manipulation of the sample without greatly increasing analytical sensitivity, fish tissues were processed with the freeze-drying/LTA method. Average sample sizes processed were 1.0 g for muscle tissue and 0.3 g for kidney and liver tissue. Thus electron microscope identification of particles in a typical grid opening of 9500 μm^2 involved 0.00004 g of muscle tissue and required ~ 1 h to complete.

Fiber concentrations for Lake Superior waters were estimated from monitoring data accumulated for different locations primarily in western Lake Superior since 1974. Only measurements made with the carbon-coated Nuclepore filter Jaffe Wick method (Cook et al. 1976; Anderson and Long 1980) were used. Chrysotile fiber concentrations for the Deception Bay, Quebec area, were used as reported (Boulva and LeBeau personal communication).

Results

Minimum detectable particle concentrations are smallest where the amount of tissue prepared and the number of fields examined were largest. The poor precision for concentrations determined when only a few fibers are identified is reflected by the large range of the 95% confidence interval calculated on the basis of a Poisson distribution for counted particles (Hallenbeck et al. 1977). Blank samples did not contain detectable amphibole fibers. For the few blank samples containing chrysotile fibers the blank concentration is calculated on the basis of the weight of the corresponding tissue sample per grid opening. Thus, the comparision of fiber concentrations in tissue and blank samples is on the basis of fibers observed in each per unit area of the grid.

Fish with a lifetime exposure to Lake Superior water were studied primarily for the presence of amphibole fibers. The amphibole fiber distribution pattern in Lake Superior (Fig. 2) results from counterclockwise circulation of fine taconite tailings discharged 11 km northeast of Split Rock, Minnesota, during the period of 1956–80 (Cook et al. 1974). Lake trout, *Salvelinus namaycush*, captured at Split Rock are thought to have a much higher exposure to amphibole fibers than lake trout captured at Huron Bay, Michigan, far from the area of extreme amphibole fiber contamination. The Split Rock lake trout are likely to have occasionally migrated out of the area of high amphibole fiber concentrations, particularly by moving to the northeast along the north shore of Lake Superior.

Amphibole fibers provide a good test of fiber accumulation by fish because they are limited in distribution in natural

TABLE 2. Fiber concentrations in fish.

Species	Location	Tissue	No. of fish	No. of prepara-tions	× weight (g)	Total fields examined	Total No. of fibers observed		Tissue concn.[a] (fibers/milligram)		Blank concn.[a] (fibers/mg)	
							Amphibole	Chrysotile	Amphibole	Chrysotile	Amphibole	Chrysotile
Salvelinus namaycush (Lake trout)	Split Rock	Muscle	2	6	1.228 (0.605−1.775)	85	5	108	1.0 (0.3−2.4)	27.0 (22.1−32.6)	<0.4 (0.0−1.5)	<0.4 (0.0−1.5)
		Kidney	2	3	0.388 (0.296−0.582)	11	202	0	1246.7 (1079.3−(1427.9)	<6.2 (0.0−12.3)	<1.7 (0.0−6.2)	<1.7 (0.0−6.2)
		Liver	2	4	0.219 (0.139−0.407)	35	6	6	20.5 (7.52−44.7)	20.5 (7.52−44.7)	<3.0 (0.0−11.0)	<3.0 (0.0−11.0)
Salvelinus namaycush (Lake trout)	Huron Bay	Muscle	1	2	1.315 (0.918−1.524)	29	2	1	1.4 (0.2−5.0)	0.7 (0.0−2.5)	<1.0 (0.0−3.7)	2.0 (0.2−7.1)
		Kidney	1	3	0.335 (0.169−0.541)	29	13	2	35.1 (18.7−60.1)	5.4 (0.7−19.5)	<3.9 (0.0−14.5)	7.8 (0.9−28.3)
		Liver	1	1	0.403	10	0	1	<6.5 (0.0−24.1)	6.5 (0.2−24.1)	<6.5 (0.0−12.1)	6.5 (0.2−24.1)
Ictalurus lacustris (Catfish)	Laboratory	Muscle	2	3	0.217 (0.078−0.494)	33	1	10	3.7 (0.1−23.2)	36.7 (17.67−67.5)	<5.5 (0.0−20.3)	11.0 (1.3−39.7)
		Kidney	1	1	0.041	6	0	0	<107.0 (0.0−394.7)	<107.0 (0.0−394.7)	<29.2 (0.0−107.6)	<58.4 (0.0−215.2)
Salvelinus fontinalis (Brook trout)	Laboratory	Muscle	1	1	0.805	6	0	2	<5.4 (0.0−20.1)	10.9 (1.3−39.3)	<3.3 (0.0−12.0)	<3.3 (0.0−12.0)
		Kidney	1	2	0.535 (0.065−0.178)	11	0	5	<25.0 (0.0−92.1)	124.8 (40.4−290.8)	<27.5 (0.0−101.3)	<27.5 (0.0−101.3)
		Liver	1	2	0.364 (0.159−0.706)	16	0	0	<5.8 (0.0−21.5)	<5.8 (0.0−21.5)	<9.3 (0.0−34.3)	<9.3 (0.0−34.3)
Salvelinus alpinus (Arctic char)	Deception Bay	Muscle	4	6	1.180 (0.850−1.868)	64	0	20	<1.4 (0.0−4.9)	2.9 (1.7−4.4)	<4.3 (0.0−15.9)	8.7 (5.2−13.6)
		Kidney	3	3	0.142 (0.103−0.272)	22.5	1	28	8.3 (0.2−30.7)	230.5 (153.3−333.1)	<9.4 (0.0−34.6)	175.9 (105.9−280.0)

[a]Measured fiber concentrations or detection limits are reported as fibers per milligram of tissue (wet weight) with a 95% confidence interval for a Polsson distribution indicated within parentheses. Blank sample concentrations or detection limits are reported as calculated for the corresponding tissue sample weight per unit area of microscope examination.

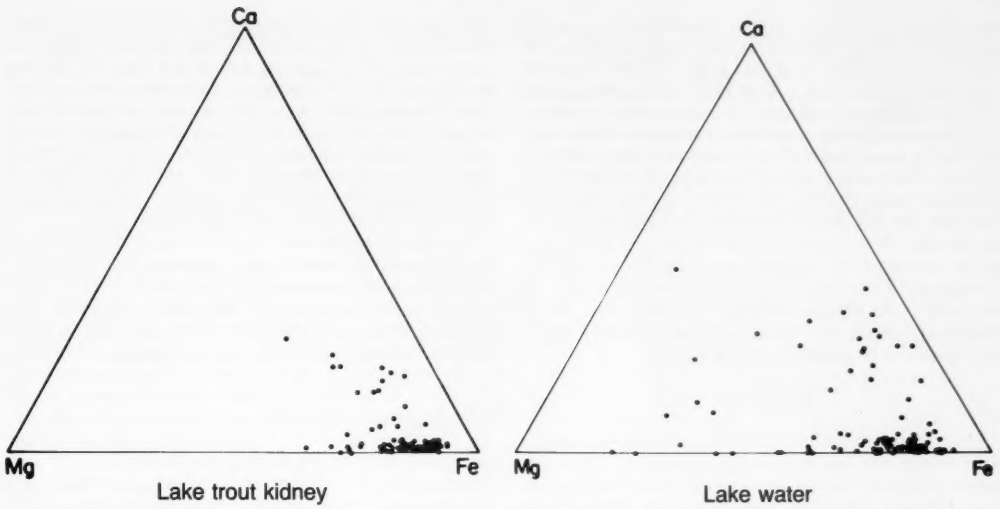

FIG. 3. Iron–magnesium–calcium ternary diagrams for the major cation compositions of 100 amphibole fibers in both Lake Superior water and kidney tissue from lake trout exposed to the water. Plotted cation X-ray emission intensity ratios are calculated directly from integrated K_α peak intensities obtained after background subtraction from the energy-dispersive X-ray fluorescence spectra of individual amphibole fibers.

waters and are not likely to present a contamination problem during analysis as are chrysotile fibers. No amphibole fibers were found in any of the blank samples analyzed. Large numbers of amphibole fibers were identified in kidney tissue of the lake trout, but only a few amphibole fibers were identified in lake trout muscle and liver tissues (Table 2). Although no difference in amphibole fiber concentration could be observed in lake trout muscle tissue for Split Rock versus Huron Bay fish, large differences existed for kidney and liver tissues. Only one amphibole fiber was found in all the tissue examined from catfish and brook trout raised in the laboratory, but only $\frac{1}{10}$ as much kidney tissue was examined for these fish when compared with the lake trout.

A check of the association between amphibole fibers in Lake Superior water and those in the lake trout tissue is provided by comparison of the elemental composition as determined by EDX analysis of individual amphibole fibers in the water and fish tissue. The unique amphibole mineral particle distribution found in Lake Superior water is also found in the fish. The major cation (iron–magnesium–calcium) compositions of 100 amphibole fibers observed both in Split Rock lake trout kidney tissue and in the lake water indicated similar amphibole mineral fiber mixtures of ~ 75% cummingtonite–grunerite $[(Fe,Mg)_7Si_8O_{22}(OH)_2]$ and 20% actinolite $[Ca_2(Fe,Mg)_5Si_8O_{22}(OH)_2]$ (see Fig. 3). The absence of aluminum in almost all amphibole particles identified precludes the presence of hornblende, a more common amphibole constituent of natural sediments and soils. The total of 15 amphibole fibers identified in Huron Bay lake trout samples fit the same amphibole mineral distribution pattern although too few fibers were identified to provide as strong an association.

Arctic char, *Salvelinus alpinus*, were collected from two locations near the mouth of the Deception River at Deception Bay, an arm of Hudson Bay in Quebec, Canada. Chrysotile fiber concentrations as high as 670 million fibers per litre in the river mouth (Boulva and LeBeau personal communication) have been measured and are attributable to a chrysotile mining operation upstream and loading of asbestos onto ships at the bay. The anadromous nature of the Arctic char and the dependence of water fiber concentrations on asbestos mining and shipping operations make it very unlikely that the fish were exposed to average chrysotile fiber concentrations exceeding 10^6 fibers/L in the year preceding their capture. This uncertain exposure is reflected in the estimated average water exposure concentration (Table 1).

Muscle tissue samples from all four Arctic char analyzed contained chrysotile fibers but more chrysotile fibers were found in blank samples (Table 2). Higher chrysotile concentrations are reported for kidney tissue, but these concentrations are only slightly greater than corresponding blank sample concentrations. Only one amphibole fiber was found in all the Arctic char samples examined.

Diatom frustule fragments were particularly common in kidney and liver tissue of lake trout and kidney tissue of Arctic char (no liver analyzed). None was observed in fish raised in the laboratory. A conservative estimate of frustule fragments was made by counting each particle with a porous, diatom frustule-like appearance. All fragments analyzed by EDX gave spectra containing only a silicon K_α peak as expected for diatom frustules which are composed of amorphous silica. The concentrations in Split Rock and Huron Bay lake trout kidney tissue were 100 and 200 particles/mg, respectively. The identification criteria prevented counting many small fragments that were undoubtedly present in the samples.

Little comparison of amphibole fiber size distributions is possible since only lake trout kidney tissue contained enough fibers to allow a good measure of the incidence of different

sizes of particles. Typical fiber size distributions in any media are characterized by large percentages of small particles. The mean amphibole fiber length and width for Split Rock lake trout kidney tissues were 1.99 and 0.37 μm as compared to 1.50 and 0.28 μm for Huron Bay lake trout kidney tissue. Lake trout muscle tissue amphibole fibers (only seven identified) had a mean length of 1.06 μm and a mean width of 0.22 μm. The kidney preparations were often heavy with debris, so many short or thin fibers may not have been observed, and this affects both fiber concentrations and size distribution. Mean amphibole fiber lengths and widths typical for western Lake Superior water are 2.0 and 0.3 μm (Cook et al. 1976); storm resuspension of settled particles and proximity to the tailings discharge may raise these values. Amphibole fibers transported to other parts of Lake Superior are expected to be primarily smaller particles.

Discussion

The primary purpose for this investigation was to determine if freshwater fish can accumulate asbestos and analogous mineral fibers and thereby act as a vehicle for human ingestion. Although only seven amphibole fibers identical with those in Lake Superior water were found in Lake Superior lake trout muscle tissue, we conclude that trace concentrations of small fibers can be present in fillets from fish exposed to mineral fibers. The concentration in muscle tissue appears to be on the order of 10^{-2} the water exposure concentration (expressed as fibers per gram). Thus the human ingestion of fish from asbestos-contaminated waters is unlikely to be a concern as in the case of drinking water supplies.

The greater number of amphibole fibers in lake trout kidney tissue may be related to the large volume of blood flowing through that organ. The possibility that the urinary tract serves as an elimination route for these small particles as demonstrated for mammals (Cook and Olson 1979) has not been investigated. The 36-fold difference in amphibole fiber concentrations for Split Rock and Huron Bay lake trout kidney tissue parallels the great difference in water fiber concentrations at the two locations. The tendency for lake trout found at Split Rock to migrate to areas with lower fiber concentrations and lake trout found at Huron Bay to migrate to areas with higher fiber concentrations may explain why the tissue fiber concentration difference is not even greater. Another factor may be the drift of food organisms into the study areas from areas of higher or lower amphibole fiber water concentrations.

The absence of amphibole fibers in the catfish and brook trout raised in amphibole fiber-contaminated water on an artificial diet suggests that ingestion of food organisms containing the fibers is the primary route for fiber accumulation in the lake trout. If so, this study provides indirect evidence for the small volume of water normally ingested by freshwater fish. We intend to expose trout to a well-measured dose of asbestos in food and compare the amount accumulated in tissue to amounts found in fish exposed only through contaminated water. The possible passage for microscopic mineral fibers through the gastrointestinal mucosa of fish and subsequent hematogenous or lymphatic transport to other tissues, particularly the kidney, is supported by reports of such a mechanism in mammals such as mice (LeFevre et al. 1978), rats (Sanders and Ashworth 1961), sheep (Nottle 1977), baboons (Patel-Mandlik et al. 1979), and man (Cook and Olson 1979). In a study of tissues of a neonate baboon that ingested asbestos, the highest fiber concentrations were found in the kidney (Patel-Mandlik 1980). A possible alternate pathway for fiber accumulation in fish, passage through gill membranes, seems to be of minimal significance since fibers were not found in fish exposed only to fibers in water. The pattern of diatom frustule fragment occurrences in tissues also supports ingestion as the primary route for particle accumulation.

Two analytical problems require further work. Techniques must be perfected which reduce chrysotile fiber contamination during tissue preparation to levels which do not interfere with the ability to detect chrysotile fibers in the tissue. The apparent association in this study of chrysotile fibers in blank samples with tissue samples having the highest concentrations of chrysotile may be due to cross contamination from the tissue tube to the blank tube in the low temperature ashing step. Amphibole fibers which are more massive than chrysotile fibers were not found in blank samples of tissues from unexposed fish. Also needed is the development of even more sensitive tissue digestion techniques to allow faster electron microscope examination of more tissue residue. Thus detection limits could be lowered and concentrations such as those reported for muscle tissue in this study could be determined with greater precision.

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Relationships between Nannoplankton and Lake Trophic Status[1]

SUSAN WATSON AND JAAP KALFF

Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Que. H3A 1B1

WATSON, S., AND J. KALFF. 1981. Relationships between nannoplankton and lake trophic status. Can. J. Fish. Aquat. Sci. 38: 960–967.

The hypotheses that with increasing eutrophication (1) nannoplankton biomass increases and (2) the relative proportion (percent) of nannoplankton biomass decreases were tested with data from Lake Memphremagog, a lake exhibiting a nutrient gradient, and on a more general scale using published data from a number of lakes. Both hypotheses were supported within and among lakes if trophic status was defined by total algal biomass. This was also generally true if total phosphorus (TP) was used as an alternative measure of trophy, although percent nannoplankton biomass showed no relationship to TP among lakes. Empirical relationships that allow a first prediction of total nannoplankton biomass from total algal biomass or TP were calculated. The data suggest that among lakes, net plankton will show a more variable relationship with TP than nannoplankton.

Key words: nannoplankton, net plankton, trophic status, total biomass, total phosphorus

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Des données recueillies dans le lac Memphrémagog, un lac à gradient d'éléments nutritifs, ont servi à vérifier les hypothèses que (1) la biomasse de nannoplancton augmente et (2) la proportion relative (pour-cent) de la biomasse de nannoplancton diminue à mesure qu'augmente l'eutrophisation. On a également fait appel, dans un contexte plus général, à des données publiées sur plusieurs autres lacs. Les deux hypothèses sont confirmées, dans un même lac et d'un lac à l'autre, quand la biomasse algale totale est utilisée pour définir la condition trophique. Il en est généralement de même aussi quand le phosphore total (PT) est utilisé comme autre mesure de l'état trophique, bien que la proportion relative de la biomasse de nannoplancton ne montre pas de relation avec le PT entre lacs. Nous avons calculé les relations empiriques permettant une première prédiction de la biomasse totale de nannoplancton à partir de la biomasse algale totale ou du PT. D'après ces données, le plancton tamisé montrerait une relation plus variable avec le PT que le nannoplancton.

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TRADITIONALLY, algal communities have been investigated at the species level, which has resulted in the accumulation of a vast quantity of taxonomic data but which has led to few general predictions about patterns within and among lakes. An alternative, simpler method is to group phytoplankton assemblages into size fractions (e.g. Pavoni 1963; Semina 1972; Kalff 1972; Munawar and Munawar 1975). Although algal taxonomic divisions are not necessarily related to function, algal physiology and cell loss rates (through sedimentation and grazing) have been related to cell size (e.g. Laws 1975; Banse 1976; Smayda 1970; Burns 1968). The division of algal communities into size fractions may therefore allow the formulation of testable hypotheses about general relationships between environmental factors and phytoplankton size distributions.

In the past, most emphasis has been placed on the larger size fractions (or net plankton) because this group is primarily responsible for nuisance blooms; moreover, it is easier to identify and count. However, the importance of smaller size fractions has become recognized since it was shown that nannoplankton frequently make sizable contributions towards total algal production and biomass (e.g. Kalff 1972; Paerl and MacKenzie 1977; Munawar and Munawar 1975), and provide a major food source for herbivorous zooplankton (Burns 1968; Gliwicz 1967, 1977). In recognition of the functional roles of size fractions, Gelin and Ripl (1978) have redefined the nannoplankton as that size fraction which is ingestible by zooplankton grazers.

Whereas one of the major consequences of increased nutrient loading to lakes is a higher overall phytoplankton standing crop (e.g. Vollenweider 1969; Dillon and Rigler 1975; Nicholls and Dillon 1978), the existence of a general quantitative relationship between nutrient levels and nannoplankton standing crop has not been established. If, as evidence suggests, nutrient uptake rates are size dependent (e.g.

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Eppley et al. 1969; Laws 1975; R. Smith and J. Kalff, McGill University, Montreal, Que., unpublished data), and nutrient limitation plays a major role in size selection, then nannoplankton should exhibit some pattern in relation to nutrient concentration. In fact, evidence from previous investigations generally suggests a decrease in the relative proportion of nannoplankton with increasing eutrophication (Pavoni 1963; Semina 1972; Gelin and Ripl 1978; Spodniewska 1978, 1979).

The purpose of this study was to quantify the relationship between nannoplankton standing crop and lake trophic status, both within one lake exhibiting a nutrient gradient (Lake Memphremagog, Quebec – Vermont) and among a number of lakes covering a wide trophic range. Trophy is here defined by total algal standing crop (Vollenweider 1969), as measured by total algal biomass. Kalff and Knoechel (1978) and Nicholls and Dillon (1978) have shown that total biomass and total phosphorus (TP) are correlated, and because nannoplankton biomass is not independent of total biomass estimates, we reexamined the relationship between these two variables for our two data sets to use them as alternative measures of lake trophy, although there are problems associated with each (see Methods section). Using both of these measures within and among lakes, we then tested the hypotheses that (1) nannoplankton biomass increases but (2) the relative proportion (percent) of nannoplankton biomass decreases with increasing trophy.

Net plankton are generally more periodic in occurrence than nannoplankton (e.g. Munawar and Munawar 1975; Reynolds 1978), and therefore mean seasonal values of net plankton biomass should be more variable. We tested the prediction that net plankton biomass will exhibit more unexplained variance in relation to TP than nannoplankton biomass, and thus that any relationship between TP and total algal biomass is mainly a result of an underlying relationship between TP and nannoplankton.

Methods

Algal standing crop is usually represented by measurements of chlorophyll *a* (chl*a*) and biomass (estimated from cell volume assuming a density of 1.0 g/cm^3; Vollenweider 1969), and although neither measurement is entirely satisfactory, we chose to use total biomass. Cellular chl*a* content fluctuates over a wide range of physical and chemical conditions (0.1 – 9.7% fresh weight; Nicholls and Dillon 1978), and nannoplankton generally have a higher chl*a* content per unit cell volume (Paasche 1960; Manney 1972; Malone et al. 1979), which may inflate estimates of their relative contribution to total algal standing crop. Furthermore, measurements of nannoplankton chl*a* usually involve fractionation by screens, which do not give consistent results (Sheldon and Sutcliffe 1969; Malone et al. 1979). With algal biomass computed from cell volume, there is error associated with estimates of mean cell volume (e.g. Willén 1976), while the proportion of noncytoplasmic cell volume may be appreciable, especially for communities dominated by net plankton assemblages (Paasche 1960; Nalewajko 1966; Sicko-Goad et al. 1977). Although some authors have used correction factors for a few taxonomic groups (e.g. Smayda 1965; Devaux 1977), the proportion of noncytoplasmic cell volume has been shown to vary both between and within most taxonomic groups (Sicko-Goad et al. 1977). Nevertheless, a significant relationship has been found between uncorrected algal cell volume and dry weight (R. Peters, McGill University, Montreal, Que., unpublished data). We used total biomass (computed from uncorrected cell volume) as an estimate of plankton standing crop because the majority of published data on net plankton and nannoplankton are reported as such.

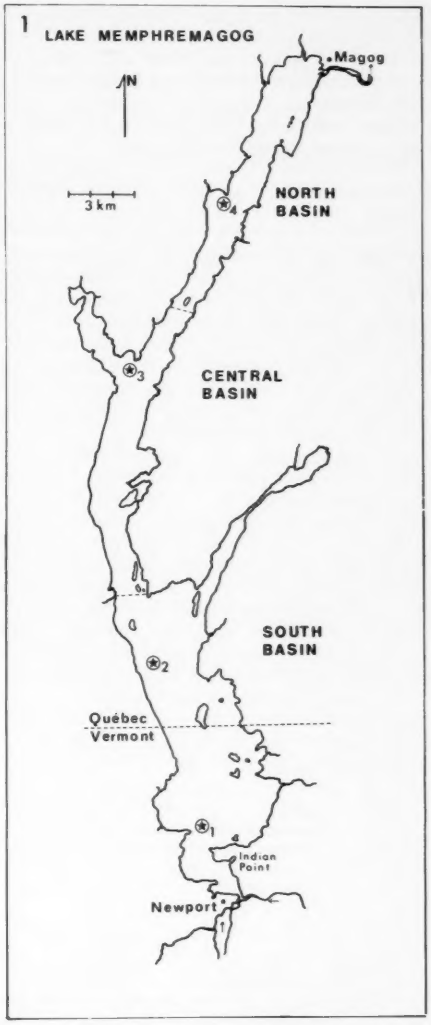

FIG. 1. Location of the four sampling stations in Lake Memphremagog.

Mean summer TP concentration was used as an alternative to total biomass to measure lake trophy. Spring overturn val-

ues may give a better prediction of summer standing crop as measured by chlorophyll (e.g. Sakomoto 1966; Dillon and Rigler 1974) but mean summer values of TP and algal biomass are more strongly correlated than similar values of chl*a* and TP (Nicholls and Dillon 1978). Furthermore, Nicholls and Dillon (1978) found that spring TP was related quite closely to average summer concentrations.

Lake Memphremagog: Nannoplankton and Trophic State

The study lake is a 40-km-long glacial lake lying on the Quebec (Canada)–Vermont (USA) border (45°06′N, 72°17′W). It can be divided morphometrically into three major basins on the basis of mean depth (Fig. 1). The lake receives an estimated 84% of its phosphorus loading from three rivers entering the southern end of the lake at Newport, VT, resulting in a north–south nutrient gradient (Peters 1979), which is also reflected in variations in primary productivity and chl*a* (Ross and Kalff 1975).

Phytoplankton samples were collected every 1 or 2 wk from May to October (1976–77) at four stations located along the phosphorus gradient (Fig. 1). Average epilimnetic phytoplankton biomass was estimated from integrated tube samples, taken above the upper boundary of the thermocline or 15 m when there was no stratification. In the shallow southern basin, samples were taken down to 0.5 m above the sediments. Phytoplankton samples were fixed with Lugol's solution and later counted using the Utermöhl technique (Vollenweider 1969). Large species of low abundance were counted under low power across diagonal transects or over half the chamber area (minimum 26% total volume settled), whereas small or common species were counted under high power across one or two diagonal transects. Replicate counts showed a fairly low variance (with ±2 SE of 6 and 16% for total and nannoplankton biomass, respectively). The maximum particle size filtered by zooplankton appears to fall below 35–40 μm (Burns 1968), and we used an upper cell dimension of 35 μm to estimate nannoplankton biomass.

We obtained TP data for Lake Memphremagog from measurements made on samples taken from 2 m in depth at the three most southern stations in 1976 and at all four stations in 1977. The samples were analyzed for TP using a persulfate digestion technique (Johnson 1971).

To test the prediction that total algal biomass is significantly correlated with TP within Lake Memphremagog, we used least-squares regression analysis both with individually matched observations and mean seasonal values calculated for each station and year.

Using both total biomass and TP as trophic measures, we analyzed both individual and mean seasonal values by least-squares regression to test our two hypotheses. To assess the degree to which part–whole correlation accounts for any correlation between total and nannoplankton biomass (Sokal and Rohlf 1969), we also performed regression analysis between the two independent estimates of net plankton and nannoplankton biomass (the former obtained by difference), again using both individual and seasonal mean values. Finally, net plankton biomass (>35 μm; obtained by difference) and TP were used in least-squares regression to test the hypothesis that net plankton show a more variable relationship with TP than annoplankton.

Among Lakes: Nannoplankton and Trophic State

To test for generality of relationships between nannoplankton and lake trophy, we searched the literature for measures of total and nannoplankton biomass and TP. The data are summarized in Table 1. The total data set is available from the Depository of Unpublished Data, CISTI, National

Table 1. Summary of the sources for data sets used for examining the relationships between nannoplankton and lake trophic status, as measured by total biomass (TB) and total phosphorus (TP).

Data source for biomass	Definition of nannoplankton (μm)	Data source for TP	No. of data points with trophic measure as: TB (individual observations) (subset A)	TP (seasonal means) (subset B)
Granberg (1970)	60	Ilmavirta and Kotima (1974)	9	1
Kalff (1972)	64	Kalff (unpublished data)	5	1
Kristiansen (1971)	45	—	31	—
Munawar and Munawar (1975)	"Phyto-flagellates"	Weiler (1978); Dobson et al. (1974); Schelske et al. (1974); DiToro and Matystik (1979)	—	14
Pavoni (1963)	30 (some filaments)	—	7	—
Spodniewska (1978)	30	—	34	—
Spodniewska (1979)	30	Spodniewska (1979)	25	—
Spodniewska et al. (1973)	20	—	13	—
Watson (1979)	35	Watson (1979)	137	—
Watson (1979)	35	Watson (1979)	—	7
Watson (1979)	35	Watson (1979)	(52)[a]	(52)[a]

[a]Winthin-lakes test only.

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Most of the authors in the literature data set delineated the upper size limits of the nannoplankton fraction as 35 μm or less, although some used other criteria (Pavoni 1963; Munawar and Munawar 1975; see Table 1).

We took two subsets of the literature data to test the relationship between nannoplankton and the two measures of trophy, total biomass and TP.

Using total biomass as an estimate of trophic level, the widest trophic range was covered using individual observations: where only seasonal values of total and nannoplankton biomass were available (Table 1) these were not included in this subset (subset A). We obtained a total of 261 data points from seven different publications (Table 1). We also included individual observations from the four stations in Lake Memphremagog.

Using TP as a trophic measure (subset B), we obtained TP from the articles themselves or estimated it from other sources (Table 1). A total of 23 data points were obtained for this subset (Table 1); no individual observations were included. Only seasonal mean estimates of TP were available for most of the lakes in these data sets (except Lake Memphremagog), and thus we tested the relationship between TP and total algal biomass only for subset B, using least-squares regression analysis. We also used regression analysis with both subsets to test our two hypotheses (see introduction). For both subsets, we used analysis of covariance (type II, SAS 1980) to determine whether the different definitions of nannoplankton, different investigators, and differences in lake morphometry (as measured by surface area and mean depth where data were available) accounted for a significant amount of the total variance in nannoplankton biomass. To test the hypothesis that netplankton has a more variable relationship with TP than nannoplankton, we regressed netplankton biomass (obtained by difference) against mean seasonal TP for subset B.

Results

Lake Memphremagog: Nannoplankton and Trophic State

Data from within Lake Memphremagog supported hypotheses 1 and 2, using both total biomass and TP as measures of lake trophy. Both least-squares (model I) and functional (model II: Bartlett's three-group method, Sokal and Rohlf 1969) regression analysis gave statistically indistinguishable results for within- and among-lake tests, and only model I results are presented here. There was no relationship between individually matched observations of these two variables within Lake Memphremagog, but seasonal mean values for each station and year yielded a significant log–log correlation (Table 2, model I).

Using total biomass as a trophic measure, we found that nannoplankton biomass showed a highly significant logarithmic increase with total biomass (Table 2, model III). The slope of this relationship was significantly less than 1.0 ($P < 0.05$), and thus the relative proportion (percent) of nannoplankton biomass decreased significantly with increasing total biomass (Table 2, model IV). Nannoplankton biomass also showed a highly significant relationship with net plankton biomass ($P < 0.0001$; Table 2, model VII).

Using TP as a trophic measure, we obtained similar results.

TABLE. 2. Summary of regression models used to examine the relationship between nannoplankton and net plankton biomass (nanB and netB, respectively) and lake trophic status, as measured by total biomass (TB) and total phosphorus (TP). (*$P < 0.05$; **$P < 0.01$; ***$P < 0.005$; ****$P < 0.001$.)

Test	n	b	(±2 SE)	a	r	p
Model I. log (TB) = a + b log (TP)						
i) Within-lake test	7	2.02	(±0.58)	1.02	0.96	****
ii) Among-lakes test	23	1.43	(±0.35)	1.47	0.88	****
Model II. TB = a + bTP						
ii) Among-lakes test	23	161.09	(±39.14)	−593.42	0.87	****
Model III. log (nanB) = a + b log (TB)						
i) Within-lake test	137	0.47	(±0.10)	1.20	0.62	****
ii) Among-lakes test	261	0.53	(±0.07)	1.03	0.70	****
Model IV. % nanB = a + b log (TB)						
i) Within-lake test	137	−35.60	(±8.19)	151.12	0.60	****
ii) Among-lakes test	241	−25.36	(±4.48)	122.61	0.58	****
Model V. log (nanB) = a + b log (TP)						
i) Within-lake test	7	0.83	(±0.56)	1.77	0.80	NS
ii) Among-lakes test	23	1.28	(±0.21)	1.24	0.93	****
Model VI. % nanB = a + b log (TP)						
i) Within-lake test	7	−72.8	(±42.24)	111.97	0.84	NS
Model VII. log (nanB) = a + b log (netB)						
i) Within-lake test	137	0.22	(±0.09)	2.06	0.39	****
ii) Among-lakes test	261	0.32	(±0.06)	1.82	0.53	****
Model VIII. log (netB) = a + b log (TP)						
i) Within-lake test	7	2.52	(±0.75)	0.29	0.95	****
ii) Among-lakes test	23	1.67	(±0.52)	0.94	0.82	****

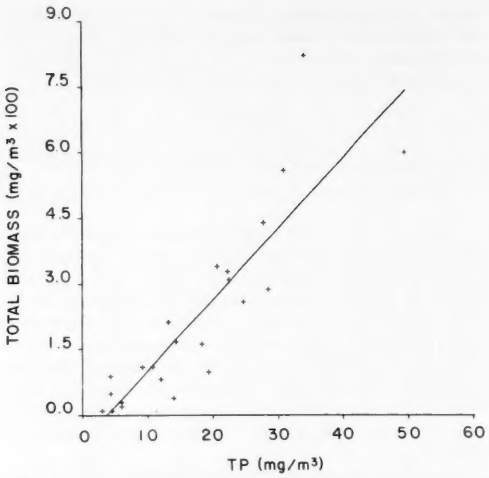

FIG. 2. Linear relationship between total biomass (TB) and total phosphorus (TP) among lakes, expressed by the equation:

$$TB = 161.09TP - 593.42.$$

As was shown above for total biomass, individual observations of both nannoplankton or net plankton biomass and TP showed no relationshp ($P > 0.05$), but seasonal mean values yielded significant positive correlations whereas there was a corresponding decrease in the relative proportion of nannoplankton biomass (Table 2, model VI). Seasonal mean net plankton biomass was more significantly correlated with mean TP than nannoplankton biomass (Table 2, models V and VIII).

AMONG LAKES: NANNOPLANKTON AND TROPHIC STATE

Data from among lakes also generally supported the hypotheses. Total biomass and TP were again highly correlated on a linear and log scale (Table 2; Fig. 2).

With total biomass as a measure of increasing trophy, nannoplankton again showed a highly significant logarithmic increase similar to that found within Lake Memphremagog (Table 2, model III; Fig. 3A):

(1) $\log (nanB) = 0.53 \log (TB) + 1.03.$

The relative proportion (percent) of nannoplankton biomass showed a corresponding decrease (Table 2, model IV; Fig. 3B). Nannoplankton and net plankton biomass were again significantly correlated (Table 2, model VII).

Using TP as a measure of increasing trophic status, there was a highly significant logarithmic increase in nannoplankton biomass (Table 2, model V; Fig. 4):

(2) $\log (nanB) = 1.28 \log (TP) + 1.24.$

However, there was no corresponding trend in percent nannoplankton biomass. Analysis of covariance showed that a small but statistically significant amount of the variance in

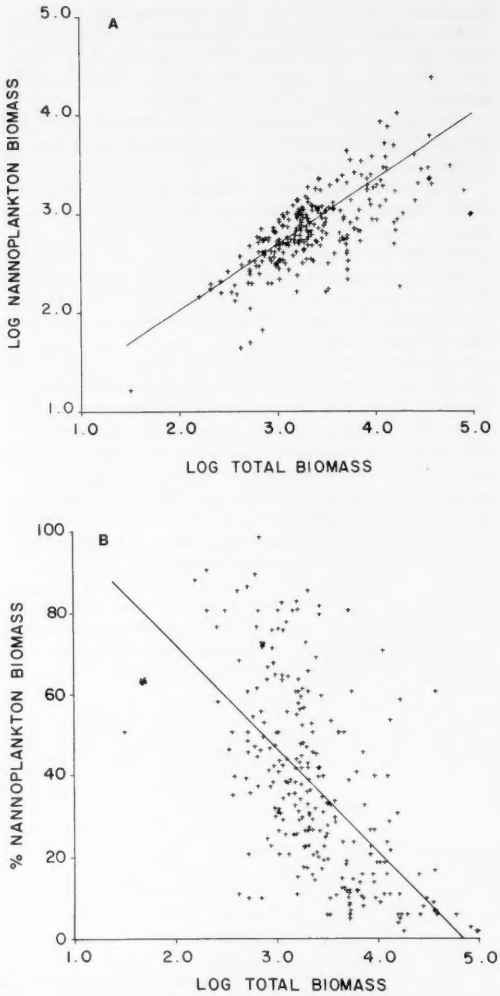

FIG. 3. General relationship between nannoplankton biomass (nanB) and total biomass (TB) among lakes, expressed by the equations:

(A) $\log (nanB) = 0.53 \log (TB) + 1.03.$
(B) $\%\ nanB = -25.36 \log (TB) + 122.61.$

nannoplankton biomass was attributable to differences between investigators and in nannoplankton size ranges used for individual observations (subset A) only (Table 3) whereas the variance accounted for by surface area and mean depth was not significant for either subset.

Net plankton biomass showed a highly significant logarithmic correlation with TP. A comparison of F values showed that TP accounted for more of the total variance in nannoplankton than net plankton biomass (Table 2).

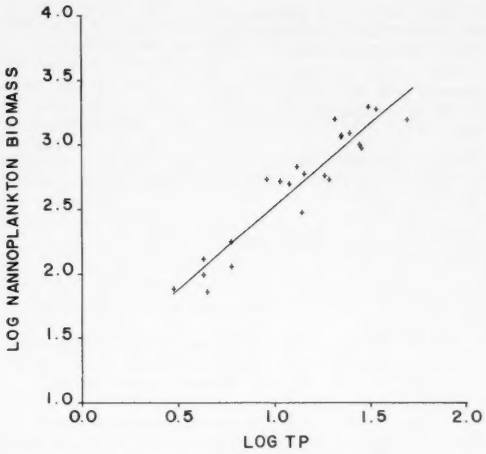

FIG. 4. General relationship between nannoplankton biomass (nanB) and total phosphorus (TP) among lakes, expressed by the equation:

$$\log (\text{nanB}) = 1.28 \log (\text{TP}) + 1.24.$$

Discussion

TP and total biomass were correlated only when seasonal means of these variables were used (Table 2, model I). Spodniewska (1979) also found no significant relationship for individual measurements of TP and total biomass for a number of Polish lakes. We therefore concluded that whereas mean seasonal TP provides an alternative to total biomass as a measure of the trophic status of lakes within our data sets, single measurements of TP do not necessarily reflect lake trophy. It is possible that the observed lack of correlation between individual measurements of total biomass and TP may be the result of a disequilibrium between phytoplankton biomass and the proportion of TP that is available (e.g. Peters 1979) and/or other size-selective factors affecting phytoplankton growth (e.g. Banse 1976) or losses through respiration, grazing, and sedimentation (Laws 1975; Banse 1976; Gliwicz 1967, 1977).

If total biomass is used as a measure of trophic state, the hypothesis that with increasing trophy there is (1) an increase in nannoplankton biomass and (2) a decrease in the relative proportion of nannoplankton biomass is supported by data within Lake Memphremagog and on a broader scale among lakes, despite the observed scatter (Table 2, model III; Fig. 3). The relationship between independent estimates of net plankton and nannoplankton biomass was highly significant both within and among lakes ($P < 0.0001$; Table 2, model VII), and we therefore concluded that the observed regressions between nannoplankton and total biomass (Table 2, model III) make valid predictions and are not simply a result of part–whole correlation. There is no question that the regressions between percent nannoplankton and total biomass involve part–whole correlation, but because the slopes of the regressions between nannoplankton and total or net plankton biomass are significantly less than 1.0, we still concluded that the relative proportion of nannoplankton biomass decreased with increasing trophy. This implies that at higher nutrient levels, either net plankton have higher growth rates or nannoplankton suffer greater losses, for example, through grazing.

There was no significant correlation between individual estimates of TP and nannoplankton biomass within Lake Memphremagog. However, we found that individual values of TP are also not a good measure of lake trophy, and the lack of correlation between these two variables does not contradict our hypotheses. Seasonal mean values of nannoplankton biomass and TP showed a significant positive correlation, both within Lake Memphremagog and among lakes (Table 2, model V), supporting the hypothesis that nannoplankton biomass increases with increasing trophy. However, the predicted corresponding decrease in the relative proportion (percent) of nannoplankton biomass was only observed within Lake Memphremagog, and was lacking among lakes. This suggests that among lakes covering a wide range of physical and chemical conditions, factors other than TP have more influence on the relative contribution of nannoplankton, whereas within the one lake we studied the factors are internally more constant and TP alone accounted for a highly significant amount of the total variance in mean seasonal percent nannoplankton biomass. Absolute nannoplankton biomass was strongly correlated with TP among lakes, and it appears that it is the net plankton which is more influenced by factors other than TP, or as we stated in our hypothesis, the net plankton have a less predictable relationship with TP than nannoplankton. A comparison of F values (Table 2: model V, $F = 8.96$ (i), 141.69 (ii); model VIII, $F = 45.56$ (i), 41.89 (ii)) shows that although this is not true within Lake Memphremagog, in general among lakes mean seasonal TP accounts for more variance in nannoplankton than in net plankton biomass. Analysis of covariance suggests that this is not an artifact of differences in technique between investigators (Table 3). Thus the general relationship between TP and total biomass (Fig. 2) is more attributable to the nannoplankton than the net plankton fraction. It is worth noting that although differences in lake morphometry did not account for a statistically significant amount of the total variance in net plankton biomass ($P < 0.053$, Table 3), mean depth accounted for more variance in net plankton than nannoplankton biomass, suggesting differential sedimentation losses.

The empirical relationships presented (equations (1), (2); Fig. 3, 4) allow a first prediction of nannoplankton biomass from total biomass or TP, which is useful for estimating trends in secondary production (McCauley and Kalff 1981). These models can eventually be refined to account for more of the unexplained variance, by incorporating other variables. Differences in technique between investigators, in the nannoplankton size ranges used, or in lake morphometry account for only a small amount of the total variance (Table 3), suggesting that the scatter around the regression lines (Fig. 3, 4) has a biological basis.

In summary, the hypotheses that with increasing trophy nannoplankton biomass increases while the relative proportion of nannoplankton decreases are generally supported by data within and between lakes. Furthermore, the empirical

TABLE. 3. Analyses of covariance (type II, SAS 1980) used to determine the relative effect of factors other than lake trophy (netB, TP) on nannoplankton and net plankton biomass (nanB, netB, respectively). Net plankton biomass is used instead of total biomass as a measure of trophy because it is independent of nannoplankton biomass. In the models presented here, the factors are nannoplankton size ranges used (size), author, and mean depth (z). ($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$; $^{****}P < 0.001$.)

Model I. log (nanB) = a log (netB) + $b$size + c log (netB) *size
Model II. log (nanB) = a log (netB) + $b$author + c log (netB) *author
Model III. log (nanB) = a log (TP) + bz + c log (TP) *z
Model IV. log (netB) = a log (TP) + bz + c log (TP) *z

	Source of variance	P		Source of variance	P
	Subset A (n = 261)			Subset B (n = 23)	
I.	Full model	****	III.	Full model	****
	Log (netB)	****		Log (TP)	****
	Size	NS		z	NS
	Log (netB) *size	NS		Log (TP) *z	NS
	R^2 = 0.395			R^2 = 0.973	
II.	Full model	****	IV.	Full model	****
	Log (netB)	****		Log (TP)	****
	Author	****		z	NS
	Log (netB) *author	NS		Log (TP) *z	NS
	R^2 = 0.417			R^2 = 0.977	

models derived from these data allow the prediction of nannoplankton biomass from two alternative measures of trophic status, total algal biomass and total phosphorus.

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Reproductive Uncertainty and the Evolution of Iteroparity: Why Don't Flatfish Put All Their Eggs in One Basket?

DEREK A. ROFF

Research and Resource Services, Department of Fisheries and Oceans, Northwest Atlantic Fisheries Center, P.O. Box 5667, St. John's, Nfld. A1C 5X1

ROFF, D. A. 1981. Reproductive uncertainty and the evolution of iteroparity: why don't flatfish put all their eggs in one basket? Can. J. Fish. Aquat. Sci. 38: 968–977.

Murphy's hypothesis that variation in reproductive life span is an adaptive response to variation in the predictability of reproductive success is examined. Murphy's contention that this hypothesis explains the variation in reproductive life span within the clupeids is reexamined incorporating further data on the Peruvian anchovy (*Engraulis ringens*). A non-significant correlation is now obtained between reproductive life span and brood strength variation; thus, the hypothesis is called into question. An alternative explanation is presented that considers the interaction between life history parameters: because it is necessary for the intrinsic rate of increase to exceed zero, variation in one parameter must be associated with variation in at least one other parameter. In the clupeids it is noted that the age of maturity and reproductive life span vary in concert and it is suggested that this provides an explanation of variation in reproductive life span. These two hypotheses are examined using data on the family Pleuronectidae, the flatfish. No correlation exists between reproductive life span and the degree of fluctuation in brood strength but there is a significant correlation between reproductive life span and age of maturity. It is concluded that variation in reproductive life span within the flatfish group is not a response to variation in reproductive success but rather a correlate of variation in age of maturity.

Key words: flatfish, Pleuronectidae, iteroparity, natural selection, reproduction, clupeids

ROFF, D. A. 1981. Reproductive uncertainty and the evolution of iteroparity: why don't flatfish put all their eggs in one basket? Can. J. Fish. Aquat. Sci. 38: 968–977.

L'article qui suit contient une analyse de l'hypothèse de Murphy à l'effet que la variation de la durée de vie reproductrice est une réponse adaptive à la variation de la prévisibilité du succès de la reproduction. L'auteur réexamine la prétention de Murphy que cette hypothèse explique la variation de la durée de vie reproductrice des clupéidés, y compris données supplémentaires sur l'anchois du Pérou (*Engraulis ringens*). La corrélation entre la durée de vie reproductrice et la variation de l'effectif d'une génération n'est pas significative, et l'hypthèse doit être remise en question. Au lieu de cela, on donne une autre explication qui tient compte de l'interaction entre les divers paramètres du cycle biologique : puisque le taux intrinsèque d'augmentation doit dépasser zéro, la variation dans un paramètre doit être associée à celle d'au moins un autre paramètre. On note chez les clupéidés que l'âge de la maturité et la durée de vie reproductrice varient de concert, et cela expliquerait la variation de la durée de vie reproductive. L'auteur examine ces deux hypothèses en faisant appel aux Pleuronectidae, les poissons plats. Il n'y a pas de corrélation entre la durée de vie reproductrice et le degré de variation de l'effectif d'une génération, mais il existe une corrélation significative entre la durée de vie reproductrice et l'âge de la maturité. En conclusion, la variation de la durée de vie reproductrice chez les poissons plants n'est pas en réponse à la variation du succès de la reproduction, mais plutôt fonction de la variation de l'âge de la maturité.

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Present address: Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Que. H3A 1B1.

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In 1954 Cole pointed out the tremendous advantage semelparity may have over iteroparity and provoked much discussion on the ecological factors likely to tip the balance in favor of iteroparity (e.g. Gadgil and Bossert 1970; Charnov and Schaffer 1973; Goodman 1974; Bell 1976). One factor that must clearly influence the age schedule of reproduction is the survival cost associated with the attempt to reproduce and the possible decrease in survival between broods due to the effort put into previous broods. Evidence for the importance of these two factors have been presented for a wide variety of organisms, examples being rotifers (Snell and King 1977), lizards (Tinkle 1969; Tinkle et al. 1970), Atlantic salmon (*Salmo salar*) (Schaffer and Elson 1975), and deer mice (Fairbairn 1977).

Another factor that may favor iteroparity is variable brood survival resulting from environmental variability (Murphy 1968; Stearns 1976; Roff 1978); this idea is illustrated by the following simple example. Consider first an animal that matures at age 1, produces 10 offspring, then dies, and suppose the probability of surviving to age 1 is either 0 or 1. The average annual rate of increase for this semelparous organism is 5. Now consider another type that also matures at age 1, has the same probability of surviving to this age (0 or 1), but which produces only five offpsring and may survive to reproduce again. The average annual rate of increase for this iteroparous organism is $2.5P/(1 - P)$ where P is the probability of the adult surviving from one breeding episode to another. If P is less than 0.66 the expected annual rate of increase of the iteroparous organism will be less than that of the semelparous type and we might initially suppose the latter to be the more "fit." However, the semelparous type has a much higher probability of leaving no offspring as both types have the same probability of brood failure per reproductive attempt. The trade-off between maximizing the average rate of increase versus minimizing its variance may tip the balance in favor of iteroparity. There is very little empirical evidence in support of the above hypothesis. Murphy (1968) suggested that a test of the hypothesis is the presence of a significant positive correlation between reproductive life span and variation in brood strength. He obtained such a correlation in the suborder Clupeoidei (herrings, sardines, etc.) and concluded that the variation in reproductive life span of this group is an adaptation to variable reproductive success. There might logically be expected a relationship between egg number and stock size, and because populations with many age groups are likely to fluctuate less than those with fewer age groups, we might also expect to find fluctuations in egg number to be less in the former type of populations. However, the relevant relationship is that between the size of the parent stock and the number of the offspring that survive to reproduce; thus, brood strength is measured when it is an index of the reproductive contribution that the offspring will make to the population. Because of the many environmental factors that can act between the egg stage and maturity any initial correlation between parent stock size and offspring numbers (measured as eggs) may be eliminated by the time the offspring reach maturity. Murphy did not test this assumption in his application of the test although it is a critical component; however, "conventional wisdom" does in fact attribute environmental phenomena as primary causes of brood fluctuations in the clupeids (drastic overfishing expected).

In this paper I examine Murphy's interpretation in the light of new data and suggest an alternative hypothesis. I then test these two hypotheses with an extensive set of data on the reproductive parameters of the family Pleuronectidae, the flatfish.

A Reexamination of Murphy's Data

In Murphy's (1968) data (Table 1), the correlation between reproductive life span and brood strength variation (measured as the ratio of maximum brood size to minimum brood size) is remarkably high ($r = 0.975$). A significant contribution to this correlation is the Peruvian anchovy (*Engraulis ringens*) which has an extremely small variation in brood strength. In 1971–72 the Peruvian anchovy population underwent a catastrophic collapse as a result of recruitment failure, despite a large parent stock (Murphy 1977). Although the exact course of this failure is not known it is believed that the occurrence of a full-scale El Niño played some role (Boerema and Gulland 1973). Thus, instead of a variation in spawning success of 2 times the factor is 11.9 times (data from Table 7 in Anon. 1973). The frequency of occurence of El Niño is approximately once every 7 yr (Boerema and Gulland 1973); hence, the collapse of 1972 may well be a regular feature. With the new estimate of recruitment success the correlation between brood strength variation and reproductive life span is reduced to 0.776, a value that is not significant at the 5% level. Furthermore, almost the entire value of the correlation coefficient is determined by one point, the Atlantic herring (*Clupea harengus*); deletion of this point reduces the coefficient to 0.115. This analysis casts doubt on the hypothesis that reproductive span in clupeids is a response to variation in spawning success.

The data presented by Murphy suggest an alternative explanation of variation in reproductive life span; note that there is a strong association between reproductive span and age at first maturity (Table 1). This suggests the hypothesis that variation in reproductive life span is a correlate of changes in the age of maturity. By this I do not imply that changes in reproductive life span are necessarily an evolutionary response to or evolutionary cause of changes in the age at maturity, but rather that there are only particular combinations of life history parameters that ensure persistence. Consider the measure of potential population growth, the intrinsic rate of natural increase, r_m, which depends upon the age schedule of survival and reproduction (Andrewartha and Birch 1954). In this paper I am concerned with organisms in which population numbers are controlled by environmental or other density-independent factors; r_m for these species will on average be zero. Suppose the age of maturity is for some reason delayed; r_m will be reduced and the population will become extinct as r_m is now less than zero. To persist with this delayed age of maturity some other life history parameter must be altered. Persistence requires an increased prereproductive survival rate, an increased reproductive output per breeding episode, or a longer reproductive life span (i.e. a higher postreproductive survival rate). Thus, two organisms whose population fluctuations are dependent upon the vagaries of environmental conditions will differ in at least two life history parameters (or none at all). A priori it is not obvious that in a group of species there should be a particular

TABLE 1. Reproduction parameters in some clupeids. Data from Murphy (1968).

Species	Reproductive span (yr)	Age at first maturity (yr)	Brood strength R^a
Engraulis ringens	2	1	2
Clupea harengus (Baltic herring)	4	2–3	3
Sardinops caerulea	10	2–3	10
Clupea harengus (North Sea herring)	10	3–4–5	9
Clupea harengus (Atlantic herring)	18	5–6	25

[a]R, ratio of maximum brood size to minimum brood size.

relationship between any two life history parameters; persistence only requires that the combination of parameters produce an r_m equal to or greater than zero. These ideas are diagramed in Fig. 1 in which I have assumed that only life history parameters that vary are age at maturity and reproductive life span. Groups of organisms with r_m equal to zero will fall on the line $r_m = 0$; all others must lie above the line. If in a group of species the age of maturity changes, then reproductive life span, or some other life history parameter, must vary.

Thus, there are two hypotheses. First, reproductive life span "may be an evolutionary response to uncertain survival from zygote to first maturity" (Murphy 1968). Second, variation in reproductive life span is a consequence of variation in other life history parameters. In the remainder of this paper I shall examine these hypotheses as they apply to pleuronectids. I selected this group for two reasons. First, the "conventional wisdom" is that there is little or no stock–recruit relationship in flatfishes (Cushing 1973; Cushing and Harris 1973) and second, there is a reasonably extensive data base for the temperate species of flatfish.

In examining the first hypothesis I have adopted Murphy's approach of correlating brood strength success with reproductive life span. However, this test is not definitive. Variation in brood strength refers to the total brood size of the population, but what is relevant from the point of view of natural selection is the predictability in survival of the brood of an individual. Although it is true that high variation in population brood success probably means high variation in individual success, low variation in population brood success may not mean low variation in individual success. If, for example, survival rates are heterogeneously distributed over time or space, then it is possible to have a fairly constant average survival rate but very unstable local survival rates. Local extinction may be very common but the statistical averaging of many patches may give little indication of this underlying variation (Roff 1974a, b).

To test definitely the hypothesis of environmental unpredictability favoring iteroparity, it is necessary to consider the distribution of survival rates rather than the average rate. Although high variation in the average rate may favor iteroparity, the effect of a stable rate can only be judged by considering the distribution of rates. However, such data are totally lacking for any species group. Although the approach used by Murphy cannot lead to a definitive rejection of the hypothesis, the degree of correlation can be interpreted as providing support for or against the hypothesis. A correlation coefficient, for example, that was very far from significance would cast doubt on the hypothesis in this instance.

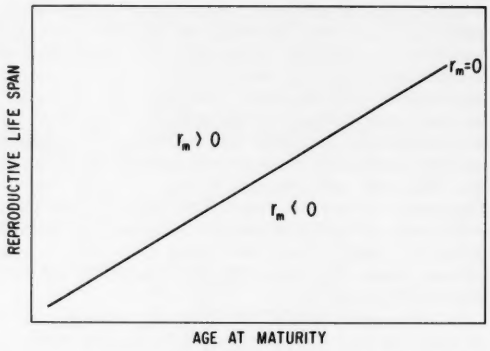

FIG. 1. Hypothetical relationship between r_m, the age at maturity, and reproductive life span.

To assess the validity of the second hypothesis, correlations between reproductive life span and other life history parameters such as fecundity or age at maturity are sought. If no correlations are found, then a more extensive analysis is in principle called for, involving the actual calculation of r_m.

Is Brood Success Correlated with Environmental Factors?

A critical component of the hypothesis that reproductive life span is an adaptation to reproductive uncertainty is the uncertainty in reproductive success. In this section I review the evidence that prereproductive survival is unpredictable in flatfishes.

I have not found any evidence indicating that in any flatfish species the size of the parent stock determines the number of offspring that survive to reproduce. There is, however, considerable evidence that in well-studied flatfishes the number of offspring is correlated with environmental factors (Table 2). I have divided the evidence into two classes; the first comprises those species in which brood success is statistically correleated with some environmental variable, and the second, those species in which a relationship has been inferred but not demonstrated.

Johansen (1927) demonstrated a significant correlation between the catch per hour of 0-group plaice, *Pleuronectes platessa*, and salinity in the Southern Kattegat and Belt Sea. From the close correspondence between plankton density and larval density he concluded "that in cold winters when there is a relatively marked outflow of comparatively fresh water from the Baltic through the Belts and Southern Kattegat, the tiny plaice larvae fail to obtain sufficient nourishment and die of starvation *en masse*."

Ketchen (1956) showed the year-class strength in British Columbian English sole, *Parophrys vetulus*, to be highly correlated with the average water temperature during the pelagic phase of this species. Based on experiments of the effect of temperature on embryonic development he hypothesized that

TABLE 2. Relationship between brood success and environmental conditions in some stocks of flatfish. *n*, number of data points; r^2, percentage of variation accounted for by the regression.

Species	Environmental variable(s)	*n*	r^2	Reference
Statistical[a]				
Pleuronectes platessa	Salinity	19	32	Johansen 1927
Parophrys vetulus	Temperature	10	82	Ketchen 1956
Parophrys vetulus	Prespawning upwelling, barometric pressure, and temperature	12	84	Hayman and Tyler 1980
Microstomus pacificus	Upwelling	17	65	Hayman and Tyler 1980
Eopsetta jordani	Barometric pressure	19	40	Ketchen and Forrester 1966
Limanda ferruginea	Temperature	22	86	Sissenwine 1974
Pseudopleuronectes americanus	Temperature	7	76	Jefferies and Johnson 1974
Hippoglossus hippoglossus	River discharge	34	64	Sutliffe 1972, 1973
Observational[b]				
Pleuronectes platessa	Temperature			Harding and Talbot 1973; Bannister et al. 1974
Solea solea	Temperature			Harding and Talbot 1973
Scophthalmus m. maeoticus	Wind			Popova 1972
Hippoglossus stenolepis	General			Anon. 1972

[a]A significant (5% or greater) correlation has been demonstrated between brood success and the environmental variable.
[b]A relationship between brood success and environmental conditions has been suggested but not statistically demonstrated.

"in years when temperatures are below average, the larvae are carried for a longer period by the northward moving currents" and "this results in a greater deposition of young on the nursery ground" (Ketchen 1956). Hayman and Tyler (1980) have proposed a similar mechanism to account for variations in year-class strength in populations of both English sole and Dover sole, *Microstomus pacificus*, off the Columbia River. The cohort strength in Dover sole is highly correlated with upwelling in early summer and offshore divergence the next winter. A more complex picture is found in the case of the English sole, cohort strength being correlated with prespawning upwelling, barometric pressure, and sea surface temperature. These factors do not, however, account for the unusually large 1961 cohort, and Hayman and Tyler suggest that this success is attributable to unique weather conditions in the winter of 1960–61. Variations in the dispersion patterns of eggs and larvae are thought to account for the variations in year-class strength of the petrale sole, *Eopsetta jordani*, population off western Canada. In this population a significant correlation can be demonstrated between year-class strength and atmospheric pressure difference (Ketchen and Forrester 1966). Using experimental data on the influence of salinity and temperature on embryonic development and assumed current values, Alderdice and Forester (1971) investigated the dispersal pattern of eggs and larvae of this species. They concluded that the hypothesis that brood strength fluctuations are due to variations in the pattern and rate of egg and larval dispersion is tenable, but that more data on the physical environment are required.

A substantial decline in catches of the southern New England yellowtail flounder, *Limanda ferruginea*, fishery during the 1940s was the result of a decrease in recruitment resulting from the adverse effects of a general warming trend in the region (Sissenwine 1974). Unfortunately the data are insufficient to suggest how abiotic factors affect recruitment in this species. Jefferies and Johnson (1974) were able to explain 76% of the variation in abundance of winter flounder, *Pseudopleuronectes americanus*, with average temperature. They suggest that the amount of temperature fluctuation is probably too small to be the direct cause of mortality, and they hypothesized that these slight changes in temperature were enough to alter the development rate of larvae thereby increasing their susceptibility to predators that migrate into the area during the summer. Sutcliffe (1972, 1973) found that the catch of Atlantic halibut, *Hippoglossus hippoglossus*, landed in Quebec is significantly correlated with the March discharge of the St. Lawrence River when moving averages of 3 yr were used with a 10-yr lag period. Sutcliffe (1972) suggested that the reason for this correlation is that runoff affects larval survival or growth.

In addition to statistical evidence linking brood success with environmental conditions, there are a number of studies that present observational evidence. Bannister et al. (1974) present evidence indicating that the year-class strength of plaice in the North Sea is established by larval mortality, and Harding and Talbot (1973) and Bannister et al. (1974) note that the very strong year-class of 1963 was associated with abnormally low temperature conditions in that year. The 1963 year-class of sole, *Solea solea*, and Atlantic cod, *Gadus morhua*, were also better than average (Harding and Talbot 1973). Christensen (1964) noted that the three strong year-classes observed in the Danish sole fishery since 1947 were "born immediately after an unusually cold winter." Popova (1972) reported that in the Black Sea turbot, *Scophthalmus maeoticus maeoticus*, there exists "a definite relationship between the yield of individual year-classes and the nature of water circulation for different directions of the wind." Much of the mortality appears to occur at the egg stage, survival of eggs being particularly low when winds exceed force 5 (Popova 1972). Severe environmental conditions in the Bering Sea during 1971 and 1972 greatly reduced the size of the 1971 year-class of Pacific halibut, *Hippoglossus*

TABLE 3. Reproduction parameters in some species or stocks of flatfish. M, instantaneous natural mortality rate; CV, coefficient of variation; R, ratio of maximum brood size to minimum brood size; N, number of years ($^*P > 0.05$).

Species	Reproductive span (yr)	Brood strength				Data sources
		M	$CV(\%)^{a*}$	R^{b*}	N	
Limanda limanda	4	—	32	2.7	14	Poulson 1933, 1937
	5.5	—	54	9.9	14	
Limanda ferruginea[c]	7	0.1	67	11.5	22	Pentilla and Brown 1973; Lux and Nichy 1969; Royce et al. 1959; Sissenwine 1977; Lux 1969
Lepidopsetta bilineata	11.5	0.26	62	11.5	19	Forrester 1969; Forrester and Thomson 1969
Solea solea	13	0.1	95	16.1	27	Houghton and Bannister 1979; de Veen 1976, 1978; Christensen 1964, 1973
Parophrys vetulus[c]	13	—	24	2.4	10	Ketchen 1947, 1956
	13	0.3	26	2.8	23	Forrester 1969; Hayman et al. 1980; Barss 1976
Microstomus pacificus[c]	14	0.2	28	2.4	12	Hayman et al. 1980; Demory 1972, 1975; A. V. Tyler, Dep. Fish. Wildl., Oregon State Univ., Corvallis, OR, personal communication
Microstomus kitt	15	—	35	3.3	16	Rae 1965
Eopsetta jordani[c]	15	—	62	8.7	18	Ketchen and Forrester 1966; Forrester 1969
Hippoglossoides platessoides	17	0.2	46	3.7	**15**	Pitt 1967, 1975, 1979
Pleuronectes platessa	26.5	0.1	69	4.9	**17**	Bannister 1978
		0.1	42	4.9	**31**	Wimpenny 1953; Cushing 1975; Anon. 1978; Beverton 1962

[a]Correlation between reproductive span and CV = 0.072.
[b]Correlation between reproductive span and $R = -0.245$.
[c]Species in which brood success has been shown to be significantly correlated to environmental conditions.

TABLE 4. Age at maturity and fecundity in some species or stocks of flatfish.

Species	Age at matuity (yr)	Size at maturity (cm)	Fecundity parameters[a]		Log (fecundity)	Data sources
			a	b		
Limanda limanda	2	12.5	0.86	3.45	4.65	Bohl 1957
Limanda ferruginea (New England)	2	32	−0.006	3.86	5.80	Howell and Kesler 1977; Royce et al. 1959
Hippoglossoides platessoides (Scotland)[b]	3	20	0.93	2.97	4.80	Bagenal 1957, 1973
Parophrys vetulus	4	34			5.63[c]	Barss 1976; Harry 1959
Microstomus kitt	4	28	1.10	2.70	5.00	Rae 1965; Newton and Armstrong 1974
Scophthalmus maximus	4.5	46			6.30[d]	Jones 1974
Pleuronectes platessa[b]	4.5	30	0.00	3.27	4.83	Wimpenny 1953; Bagenal 1973
Limanda ferruginea (Newfoundland)	6.4	37	−1.45	4.69	5.93	Pitt 1970
Pseudopleuronectes americanus	6.5	25	−0.45	4.06	5.23	Kennedy and Steele 1971
Limanda aspera[b]	8	28	−0.37	3.71	5.74	Tikhonov 1977
Glyptocephalus cynoglossus	8.6	50	−0.36	3.41	5.44	Bowering 1976, 1978
Microstomus pacificus	9	38	−1.240	3.73	6.65	Demory 1975; Harry 1959; Tyler personal communication
Hippoglossoides stenolepis[b]	11	120	−1.59	3.16	4.98	Schmitt and Skud 1978
Hippoglossoides platessoides (Newfoundland)	15	40.2	0.32	3.17	5.71	Pitt 1964, 1966

[a]Log (fecundity) = $a + b$ log (length)
[b]Several years given: one representative year chosen.
[c]Taken from figure because not all data presented.
[d]Taken from figure because parameters presented in paper apparently in error.

TABLE 5. Age at maturity and reproductive span (years) in some species or stocks of flatfish.

Species	Age at maturity	Reproductive span	Data source
Cynoglossus semifasciatus	1	1	Seshappa and Bhimachar 1953, 1954
Limanda limanda (Kattegat)	2	4	Poulsen 1933
(North Sea)	2	6	Bohl 1957
Limanda ferruginea (New England)	2	7	Lux and Nichy 1969; Royce et al. 1959
Limanda limanda (Belt Sea)	2.5	5.5	Poulsen 1933
Platichthys stellatus	2.5	5.5	Orcutt 1950
Citharichthys sordidus	3	5	Arora 1951
Pseudopleuronectes americanus (New England)	3	4	Bigelow and Welsh 1924; Perlmutter 1947; Poole 1966
Isopsetta isolepsis	3	8	Kutty 1963; Forrester 1969
Hippoglossoides platessoides (Scotland)	3	3	Bagenal 1955a, b. 1957
Solea solea	3.2	13	de Veen 1976, 1978; Christensen 1964, 1973
Limanda herzensteini	3.5	4.5	Wada 1970a, b
Lophosetta aquosa	3.5	4.5	Moore 1947
Microstomus kitt	4	15	Rae 1965
Parophrys vetulus (Oregon)	4	13	Barss 1976
(British Columbia)	4	13	Ketchen 1956; Forrester 1969
Pleuronectes platessa	4.5	26.5	Wimpenny 1953; Cushing 1975
Scophthalmus maximus	4.5	33.5	Jones 1974
Lepidopsetta bilineata	4.5	10.5	Forrester 1969; Forrester and Thomson 1969
Limanda ferruginea (Newfoundland)	6.4	5.5	Pitt 1970, 1974
Hippoglossoides ellassodon	6.5	5.5	Mineva 1964
Pseudopleuronectes americanus (Newfoundland)	6.5	6.5	Kennedy and Steele 1971
Eopsetta jordani	8	15	Ketchen and Forrester 1966
Limanda aspera	8.5	9.5	Fadeev 1970
Glyptocephalus cynoglossus	8.6	14.5	Bowering 1976
Microstomus pacificus	9	14	Demory 1972, 1975; Tyler personal communication
Hippoglossus stenolepis	11	16	Schmitt and Skud 1978
Hippoglossus hippoglossus	12	17	McCracken 1958
Hippoglossus platessoides	15.2	17	Pitt 1966, 1973

stenolepis (Anon. 1972), though the long-term decline in year-class size since the 1940s does not appear to be the result of a long-term change in the environment (Hoag and McNaughton 1978).

Is Reproductive Life Span Related to Brood Strength Variation?

Reproductive life span is an extremely tenuous measure unless the age schedule of survival rates is well established. Unfortunately there are no marine fish for which this is the case. I have attempted to estimate the reproductive life span by taking the difference between the maximum age and the age at first reproduction, defined as that age at which 50% of females are mature. Obviously the larger the sample the greater is the probability of finding an abnormally old fish. Estimates of life span are based on periods prior to heavy fishing. Because of the variety of data sources the decision on the maximum age is subjective, but the gross pattern is reliable, it being quite clear, for example, that dab, *Limanda limanda*, do not live as long as American plaice, *Hippoglossoides platessoides* (Table 3). The estimates of the age at which 50% of the females are mature are subject to much less error and are quite reliable. Also shown in Table 3 are the published estimates of instantaneous natural mortality. Quite clearly there is little relationship between these estimates and the estimated reproductive life span. Natural mortality rates will be a better measure of reproductive life span than the first method of estimation only if the rate is constant with age and the statistical methods of estimation are satisfactory. The former is unlikely to be true (Beverton and Holt 1959) and the latter very difficult to achieve given the rather small differences in survival rate. The problems are most clearly seen in the case of *L. ferruginea* which rately exceeds 9 yr in age (Royce et al. 1959; Lux and Nichy 1969; Penttila and Brown 1973), but which has been given a natural mortality rate of 0.1 (although Lux (1969) estimates it as 0.2). The discrepancy between the maximum age and natural mortality rate may be explained in part by a high mortality rate in the first 3 yr of life (Sissenwine 1977). Whatever the reason the value of 0.1 obviously does not reflect, by itself, the potential reproductive life span of yellowtail flounder.

Murphy (1968) measured the variation in spawning success in clupeids by the ratio, highest to lowest. I have calculated

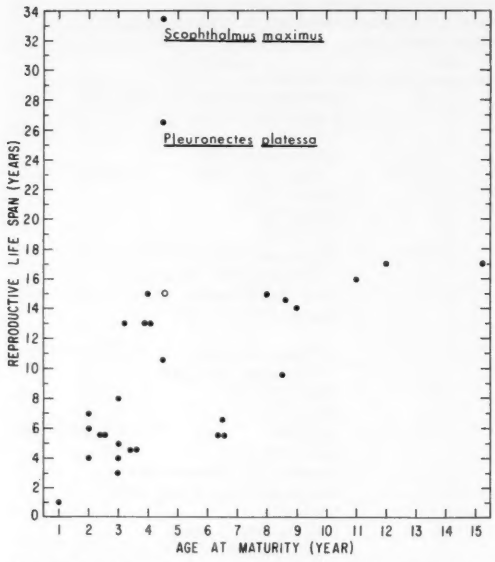

FIG. 2. Reproductive life span versus age at maturity. Open circle, adjusted reproductive life span of *Scophthalmus maximus*.

both this ratio and the coefficient of variation. Brood strength measures are based on either cohort analysis or catch per unit effort statistics, and the number of years range from 10 to 31. There is no evidence that the longer series are more or less variable than the shorter. In the case of *P. vetulus* the brood strength measures were estimated for stock relatively close together (Oregon and British Columbia) using in one case cohort analysis data (Hayman et al. 1980) and the other catch per unit effort data (Ketchen 1956). The close agreement between the two sets of data (Table 3) suggests that the particular method of assessing brood strength is not critical.

In the five populations of clupeids examined by Murphy (1968) (Table 1) the variation in brood strength ranges from 2 to 25; the range presented in Table 3 is almost as large, from 2 to 16. The reproductive spans in the two studies are also similar, in Murphy's from 2 to 18 and in the present study from 4 to 26.5. However, in the present case there is no evidence of any relationship between reproductive life span and variation in brood strength (Table 3). *Limanda limanda* and *L. ferruginea* have the shortest reproductive span but also relatively large variation in brood strengths, whereas *P. platessa* and *H. platessoides* are notable for their very long lives and the small variation in brood strength. These results hold whichever measure ofd brood strength is adopted. In 5 of the 11 species listed in Table 3 a significant correlation has been found between brood success and environmental conditions (see Table 2). For these species there is no evidence that reproductive lifespan increases with variation in brood strength. The number of years is admittedly small when considering evolutionary events, but the complete lack of even a trend suggests that the brevity of the time series is not responsible.

Is Reproductive Life Span Correlated with Other Life History Parameters?

As outlined earlier, the three likely parameters to be correlated with reproductive life span are average prereproductive survival, fecundity, and age of maturity. Data on prereproductive survival rates are insufficient to attempt an analysis. In most cases fecundity (Table 4) is more closely related to size than age; hence, fecundity was determined using the length at which 50% of females are mature. The logarithm of fecundity is used rather than fecundity because r_m is approximately proportional to log (fecundity) (Andrewartha and Birch 1954). There is no relationship between log (fecundity) and age at maturity ($r = 0.106$, $P > 0.05$).

However, there is a significant correlation between reproductive life span and age at maturity (Table 5, Fig. 2, $r = 0.429$, $n = 29$, $P < 0.05$). Two species significantly reduce the correlation, *Scophthalmus maximus* and plaice, *P. platessa* (omitting these two $r = 0.699$, $P < 0.01$). These two species have no more biological similarity to each other than to the remaining species (the turbot is principally piscivorous (Rae and Devlin 1972), whereas the plaice feeds mainly on invertebrates (Wimpenny 1953; Lande 1973)) and, thus, there is no obvious reason why they should be so different from the rest. Rae and Devlin (1972) suggested that the method of preparing the otoliths in turbot for age reading may lead to overestimates of the maximum age, and on the basis of a combination of methods they found no females older than 19 yr of age. This would reduce the reproductive life span of the turbot to 14.5 yr, a value more in line with other species. Plaice, however, do appear to have long lives. This fact may account for the high abbundance of plaice and their ability to withstand very high fishing pressure over the last 50 yr (Gulland 1968).

Discussion

In flatfish there is no relationship between brood strength variation and reproductive life span. Because the measure of brood strength variation refers to population rather than individual success, the hypothesis that reproductive life span is an adaptation to unpredictable reproductive success cannot be rejected on this basis alone. However, the complete lack of even a trend suggests that the hypothesis is unlikely to be correct. Furthermore, the existence of a significant positive correlation between age at maturity and reproductive life span provides an alternative explanation for the variation in the latter parameter. The same pattern is found in the clupeid species cited by Murphy (1968); the addition of further data on brood strength variation for the Peruvian anchovy reduces the correlation between reproductive life span and reproductive success to an insignificant value. There is also a marked trend for the reproductive life span to increase with the age of maturity. Leggett and Carscadden (1978) hypothesized that reproductive life span in American shad (*Alosa sapidissima*) is a response to variability in recruitment. But in this species fecundity and repeat spawning are highy correlated though the age at maturity is uncorrelated with reproductive life span (Leggett and Carscadden 1978).

In comparison with other groups of marine fishes the flatfishes tend to be long-lived. The evidence on reproductive success indicates that this is generally controlled by environmental factors. It therefore seems likely that the average reproductive life span of the group is a response to unpredictable reproductive success. In this sense the flatfish support Murphy's hypothesis. Within the group other factors are more important and the between-species variation is associated with variation in age at maturity rather than unpredictable reproductive success.

In presenting the second hypothesis examined in this paper I have not presumed any strict causal relationship between changes in the age at maturity and reproductive life span. That such a relationship exists in both the clupeids and flatfishes suggests that the particular combinations of life history parameters observed are not a random collection constrained only by the requirement that r_m exceeds zero. I defer an examination of such mechanisms to a later paper.

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NOTES

Release of Phosphorus by Certain Benthic Invertebrates[1]

WAYNE S. GARDNER, THOMAS F. NALEPA, MICHAEL A. QUIGLEY, AND JOHN M. MALCZYK

U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, 2300 Washtenaw Avenue, Ann Arbor, MI 48104, USA

GARDNER, W. S., T. F. NALEPA, M. A. QUIGLEY, AND J. M. MALCZYK. 1981. Release of phosphorus by certain benthic invertebrates. Can. J. Fish. Aquat. Sci. 38: 978–981.

Phosphate release rates by *Stylodrilus heringianus*, tubificids, and *Chironomus* spp. were quantified in laboratory experiments by incubating the animals in wet sand under two temperature regimes (5 and 20°C) and under two nutritional states (full and empty guts). Inorganic phosphorus release rates (±SE) for animals incubated 24 h ranged from 0.12 ± 0.02 (n = 5) nmol phosphorus (P)·(mg ash-free dry weight)$^{-1}$·h^{-1} for *S. heringianus* beginning with cleared guts at 5°C to 0.81 ± 0.09 (n = 5) nmol P·(mg ash-free dry weight)$^{-1}$·h^{-1} for chironomids beginning with full guts at 20°C. Calculations based on total invertebrate biomass and mean basal release rate suggest that benthic invertebrate excretion could account for most P released from aerobic Lake Michigan sediments.

Key words: phosphorus, benthic invertebrates, macroinvertebrates, excretion, nutrients, sediments, nutrient release

GARDNER, W. S., T. F. NALEPA, M. A. QUIGLEY, AND J. M. MALCZYK. 1981. Release of phosphorus by certain benthic invertebrates. Can. J. Fish. Aquat. Sci. 38: 978–981.

Des expériences en laboratoire au cours desquelles les sujets ont été incubés dans du sable mouillé à deux régimes de température (5 et 20°C) et à deux états nutritifs (tube digestif plein et vide) nous ont permis de quantifier les taux de mise en liberté du phosphate chez *Stylodrilus heringianus*, des tubificides et *Chironomus* sp. Les taux (±ET) de libération du phosphore inorganique chez des animaux incubés pendant 24 h varient entre 0,12 ± 0,02 (N = 5) nmol de phosphore (P)·(mg de poids sec sans les cendres)$^{-1}$·h^{-1} chez *S. heringianus* dont le tube digestif est vide au début à 5°C et 0,81 ± 0,09 (N = 5) nmol de P (mg de poids sec sans les cendres)$^{-1}$·h^{-1} chez les chironomides dont le tube digestif est plein au début à 20°C. Des calculs fondés sur la biomasse totale d'invertébrés et les taux moyens de base de mise en liberté suggèrent que l'excrétion par les invertébrés benthiques pourrait expliquer la plus grande partie du P libéré des sédiments aérobies du lac Michigan.

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ALTHOUGH a number of studies have quantified phosphorus (P) release rates of pelagic invertebrates (e.g. Pomeroy et al. 1963; Barlow and Bishop 1965; Hargrave and Geen 1968; Peters and Rigler 1973; Ferrante 1976; Lehman 1980) and benthic marine invertebrates (Johannes 1964b; Kuenzler 1961), the amount of P released by freshwater benthic invertebrates remains largely unknown. Mineralization of detritus P by benthic animals may contribute to nutrient recycling in lakes and should be quantified (Nalepa et al. 1980). Nutrient regeneration by these animals may result from their direct use of nonliving organic material or, more probably, from use of microbial biomass ingested with the material (e.g. Giere 1975; Tenore and Hanson 1980).

In recent microcosm studies, the activities of chironomid larvae (and possibly tubificid worms) significantly increased the release of P from sediments into overlying waters (Gallepp et al. 1978; Gallepp 1979; Graneli 1979; Holdren and Armstrong 1980. However, these investigations did not establish whether the increased release of P resulted mainly from physical translocation (bioturbation) or from biological release via digestive and excretory processes. Gallepp (1979), using a regression equation developed for marine animals (Johannes 1964a), calculated that chironomid excretion of P could account for all of the P released from the sediments

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during his microcosm experiments. This implies that digestive and metabolic processes of animals may be important mechanisms for mineralization of P in sediments.

The purpose of this study was to measure biogenic P release by some common benthic invertebrates, thereby providing an estimate of P release apart from the effects of bioturbation and sediment–P interactions. Direct chemical determination of P release by animals in natural sediments was not feasible because phosphate is readily adsorbed by sediments (Syers et al. 1973); yet, substrates are required by benthic organisms for normal metabolism (Provini and Marchetti 1976; Vernberg et al. 1977). We overcame these problems and quantified P release rates of some common benthic organisms by incubating the animals in wet sand substrates and extracting P from the sand with 0.1 mol/L NaOH after the animals were removed.

Methods — Benthic organisms were collected from two sites in southeastern Lake Michigan. Site 1 was located in Lake Michigan about 10 km west of the mouth of the Grand River at a water depth of 45 m. Sediments at this site consisted of silt with some sand. The oligochaete fauna at this site consisted almost entirely of *Stylodrilus heringianus*. Site 2 was located in the Grand River about 1 km upstream from the mouth at a depth of 7 m. The sediments here consisted of silt and sandy silt and the oligochaete fauna were mostly tubificids of the type without hair setae, probably of the genus *Limnodrilus*. Chironomids from this site were identified as being mainly *Chironomus* anthracinus-gr. with some *C. fluviatilis*-gr. and *C. plumosus*. A series of Ponar grab samples were taken at each site and the sediments washed through a 0.6-mm screen. Retained animals were transferred to the laboratory in natural sediment substrate and maintained in unlighted, aerated aquaria at 5 and 20°C for at least 24 h before experiments were begun. Animals from site 2 were divided and incubated at both 5 and 20°C to approximate natural seasonal temperature extremes. *Stylodrilus heringianus* collected in July 1980 from the offshore site 1 were incubated only at 5°C because sediment temperatures at that depth do not change substantially with season. Tubificids were collected from site 2 in August and October and chironomids in July and September 1980.

Release rates were measured over both long-term (24 h) and short-term (30 min) incubations. The former were conducted in linear polyethylene (to allow oxygen exchange) scintillation vials containing 5 g of washed and ignited sand (Mallinckrodt, catalog No. 7062; median grain size = 0.32 mm diameter) and 9 g of low-P purified distilled water (PDW). The sand and vial were rinsed five times with PDW and additional water was then added to give a total weight of sand plus water of 14 g. Prepared vials were placed in the test incubator overnight for temperature equilibration. Seven to 11 worms were added to each test vial for oligochaete experiments whereas chironomid experiments were conducted with two or three animals per vial. Control vials contained sand and water but no animals. All animals were cleaned of adhering debris and then rinsed thoroughly with distilled water in a beaker before they were placed in test vials. The animals began burrowing into the sand substrate almost immediately. Incubations were terminated after 24 h by removing the animals from the vials with clean forceps. Results from test vials with dead or missing organisms were not used for rate calculations. Long-term experiments were conducted both with animals taken directly from the culture aquaria (full guts) and with animals that had been placed in PDW for 18 h before incubations were started (cleared guts).

Phosphorus was extracted from the sand of the long-term experiments by adding 1 mL of 1 mol/L NaOH to each vial and horizontally mixing the closed vial at ambient temperature on a rotary shaker at 275 rpm for about 18 h. Extraction of particulate materials with dilute NaOH solution removes loosely bound phosphates (Sagher 1976). About 5 mL of extract was centrifuged for 1 h at ~1500*g* and analyzed for P with a Technicon AutoAnalyzer method (Fishman and Skougstad 1965) modified to include continuous sample digestion by hydrogen peroxide–ultraviolet light oxidation and acid hydrolysis with 0.15 mol/L sulfuric acid for 12 and 15 min sequentially. Two-tenths millilitres of each sample was injected into the analytical system with an Altex liquid chromatographic sampling valve. Samples also were analyzed without expsoure to ultraviolet light and hydrogen peroxide to differentiate between inorganic P (P_i) and ultraviolet light labile dissolved organic P (P_o). Recoveries of P from known organic P compounds by this ultraviolet light oxidation technique ranged from 35% for the relatively refractory phytic acid to 95% for glucose 6-phosphate. Standard solutions of P were prepared in 0.1 mol/L NaOH to make them comparable to sample extracts. The mean P concentration in control vials of a given experiment was subtracted from the concentration of P in each test vial to estimate the amount of P released by the invertebrates in the test vial over the incubation interval. Analysis of some sample extracts (chironomid experiments) without acid hydrolysis indicated that soluble reactive P (Fishman and Skougstad 1965) accounted for all of the inorganic P in the sample extracts.

The short-term experiments were conducted on individual tubificids. Each animal was removed from the culture aquaria and placed in a 4-mL glass vial with 0.26 g washed sand and 1.8 mL PDW. After a 30-min incubation, the animal was transferred to a second sand–water vial for another 30-min incubation. After the animal was removed, 0.2 mL of 1 mol/L NaOH was added to each of the two experimental vials and to control vials and the P was extracted from each as in the long-term experiments. Short-term experiments were conducted only at 5°C.

Animals from each experimental vial were dried in aluminum planchets for 24 h at 70°C and then ashed for 1 h at 550°C to determine ash-free dry weight (AFDW). Mean AFDW for oligochaetes was 0.66 mg per individual as compared with 1.21 mg per individual for chironomids.

Results and discussion — We observed measurable inorganic P (P_i) release for all three taxa of benthic invertebrates examined (Table 1). Mean weight-specific P_i release rates for the long-term experiment ranged from 0.1 to 0.8 nmol P·(mg AFDW)$^{-1}$·h^{-1}. With one exception, the standard errors for replicate treatments were less than 20% of the mean. Although ultraviolet light labile organic P (P_o) was observed in most test extracts, quantities were lower and more variable than P_i levels and no particular patterns of release could be defined for the different species or experimental conditions (Table 1).

Because this is, to our knowledge, the first study quan-

TABLE 1. Mean weight-specific phosphorus release rates (±SE) during 24-h incubations in wet sand for macroinvertebrates collected from southeastern Lake Michigan (site 1) and the mouth of the Grand River (site 2). FG = full gut at beginning of incubation; CG = cleared gut; n = number of replicate incubation vials. See text for further experimental details.

Organism	Collection site and time of collection 1980	Treatment, temp. of incubation (°C)	Phosphorus release rates (nmol P·(mg AFDW)$^{-1}$·h^{-1})					
			Inorganic			Organic		
			$\bar{x}$	SE	(n)	$\bar{x}$	SE	(n)
Chironomids	Site 2, Sept.	FG, 20	0.81	0.09	(5)	0.15	0.06	(5)
Chironomids	Site 2, Sept.	CG, 20	0.68	0.10	(5)	0.21	0.09	(5)
Chironomids	Site 2, July	FG, 5	0.57	0.10	(4)	0.10	0.01	(4)
Chironomids	Site 2, Sept.	CG, 5	0.37	0.06	(5)	0.05	0.03	(5)
Chironomids	Site 2, Sept.	FG, 5	0.27	0.11	(4)	0.00	0.01	(4)
Stylodrilus	Site 1, July	FG, 5	0.27	0.03	(5)	0.17	0.07	(5)
Tubificids	Site 2, Aug.	CG, 20	0.24	0.03	(5)	0.10	0.02	(5)
Tubificids	Site 2, Aug.	FG, 20	0.19	0.02	(6)	0.12	0.01	(6)
Tubificids	Site 2, Aug.	CG, 5	0.17	0.03	(5)	0.01	0.01	(5)
Tubificids	Site 2, Aug.	FG, 5	0.16	0.02	(5)	0.07	0.02	(5)
Stylodrilus	Site 1, July	CG, 5	0.12	0.02	(5)	0.02	0.01	(5)

tifying P release rates for these benthic organisms, no comparisons with previous data can be made. The measured release rates from our long-term experiments were 2 to 100 times lower than most rates reported for planktonic invertebrates (Pomeroy et al. 1963; Barlow and Bishop 1965; Hargrave and Geen 1968; Martin 1968; Ganf and Blazka 1974; Ferrante 1976; Lehman 1980). These lower rates may be expected considering the inverse relationship between weight-specific P excretion rate and size (Johannes 1964a; Hargrave and Geen 1968). Our data compare favorably with calculated basal rates obtained from the animal dry weight: weight-specific P release rate relationship reported for marine crustaceans (Hargrave and Geen 1968). The only invertebrate of comparable size (~1 mg AFDW) for which P excretion rates have been previously measured was a marine amphipod (Johannes 1964b). The P_i excretion rate for this animal under starved conditions was 1.67 nmol·(mg AFDW)$^{-1}$·h^{-1}.

The mean P_i release rates for chironomids (combining all temperature regimes and nutritional states) was significantly greater ($P < 0.01$, Mann–Whitney U-test) than the mean release rate for oligochaetes. This difference is likely related to the higher metabolic rate of chironomids. Although chironomids are larger, their weight-specific respiration rates are twice those of oligochaetes (Johnson and Brinkhurst 1971).

Except for *S. heringianus*, there were no significant differences in P_i release rates between animals with full guts and those with cleared guts during the long-term experiments (Table 1). This could indicate either that release rates were not decreased by removing the animals from food or that the effects of feeding were relatively short-lived and were not differentiated in experiments with long (24 h) incubations. Because studies with planktonic invertebrates (Peters and Rigler 1973; Lehman 1980; Gardner and Scavia 1981) and marine benthic animals (Johannes 1964b) indicate that feeding animals excrete more nutrients than animals deprived of food for several hours, we hypothesized that the similar rates between animals with full guts and empty guts may have been due to masking of early elevated rates by the long incubation times. To test this hypothesis, we carried out short-term P release experiments on individual tubificids at 30-min intervals. Not including two exceptionally high values (38 nmol P_i·(mg AFDW)$^{-1}$·h^{-1} and 86 nmol P_o·(mg AFDW)$^{-1}$·h^{-1}), the mean release rates (± SE, n = 5) for the first 30-min incubations were 3.34 ± 1.78 nmol P_i·(mg AFDW)$^{-1}$·h^{-1} and 3.59 ± 4.06 nmol P_o·(mg AFDW)$^{-1}$·h^{-1}. The second 30-min incubations yielded mean release rates (± SE, n = 6) of 1.07 ± 0.22 nmol P_i·(mg AFDW)$^{-1}$·h^{-1} and 0.75 ± 0.20 nmol P_o·(mg AFDW)$^{-1}$·h^{-1}. These P_i and P_o release rates were more than an order of magnitude greater than corresponding P_i and P_o release rates measured in 24-h incubations. P_o constituted a greater proportion of the total P in the short-term experiments than in the long-term ones. This probably reflected passage of P_o through the gut soon after the animals were removed from food. The 24-h experimental results may reflect mainly basal rates because the effects of rapid release due to digestive processes were diminished as a result of the longer incubations. Release rates from our 24-h experiments, therefore, provide conservative estimates of P release. Release rates based on our short-term experiments reflect release under more natural conditions (constant food source) but may be biased upward because they include the release of ingested nutrients that have passed through the gut unchanged.

Temperature effects on release rates in the long-term experiments were not as great as expected. Although the P_i release rates of both chironomids (collected in September) and tubificids were significantly greater at 20 than at 5°C (Mann–Whitney U-test, $P < 0.05$, one-tail, combining gut states for each taxa), the differences, particularly for tubificids, were far below those expected based on a Q_{10} of 2 (Table 1). Also, the mean P_i excretion rate of chironomids collected in July and incubated at 5°C was not significantly different from that of chironomids collected in September and incubated either at 5 or 20°C. Further experiments are needed to form definitive conclusions about temperature or seasonal effects on P release by these benthic invertebrates.

The significance of P release by benthic invertebrates can be put into perspective by comparing excretion rates to the flux of P across the sediment–water interface. Aerobic sediments from intact cores, collected at a station in southeastern

Lake Michigan (water depth = 11 m) and incubated at 12°C, released P into overlying water at a rate of 0.029 nmol $P \cdot cm^{-2} \cdot h^{-1}$ (SE = 0.003, $n = 45$; M. A. Quigley, National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, Ann Arbor, MI 48104, USA, unpublished data). The mean respective AFDWs of tubificids and chironomids at this station were 0.031 and 0.007 mg $AFDW \cdot cm^{-2}$ (data from Nalepa and Quigley (1981) with AFDW assumed to be 90% of dry weight). These biomass estimates represent 22 and 5% of the total invertebrate biomass collected at this station. Based on these biomass measurements and on our mean 24-h release rates, P excretion by tubificids and chironomids represented 20 and 13% of the total P released from these sediments. If the assumption is made that other benthic invertebrates release P at rates similar to those of tubificids and chironomids, invertebrate excretion could account for virtually all of the P released from these aerobic sediments and must be considered an important mechanism for nutrient regeneration in lakes.

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A Method for Comparing the Precision of a Set of Age Determinations

R. J. BEAMISH AND D. A. FOURNIER

Department of Fisheries and Oceans, Resource Services Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

BEAMISH, R. J., AND D. A. FOURNIER. 1981. A method for comparing the precision of a set of age determinations. Can. J. Fish. Aquat. Sci. 38: 982–983.

An index of average percent error is a better estimate of the precision of age determinations than the conventional percent agreement method because it is not independent of the age of a species.

Key words: age determination, aging errors

BEAMISH, R. J., AND D. A. FOURNIER. 1981. A method for comparing the precision of a set of age determinations. Can. J. Fish. Aquat. Sci. 38: 982–983.

Un indice du pourcentage moyen d'erreur constitue une meilleure estimation de la précision de la détermination de l'âge que la méthode classique du pourcentage convenu, car il dépend de l'âge d'une espèce.

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MANY methods have been developed to compare the precision of age determinations. One of the more common techniques is to compare the percent of determinations that are in agreement within a specified number of years. However, a percent agreement technique does not evaluate the degree of precision equally for all species. For example, if 95% of age determinations between two readers agree within ± 1 yr for Pacific cod (*Gadus macrocephalus*), this can be very poor precision since most commercial samples contain only a few year-classes (Kennedy 1970). Similarly, if 95% of spiny dogfish (*Squalus acanthias*) age determinations agree within ± 5 yr, this can represent very good precision since dogfish may be as old as 60 yr with approximately 30 age groups in a fishery (Wood et al. 1979). The use of an index that is not independent of age would provide a better estimate of precision than the percent agreement technique.

The word precision is used to describe the reproducibility of age determinations. It does not imply that the age estimates are accurate and only relates to the consistency among determinations.

Method — An average percent error can be calculated for a repetitive series of determinations (either by the same reader or by different readers) and compared to any number of other determinations. The calculation is simple and can be defined as follows:

N fish are aged; R is the number of times each is aged. Let X_{ij} be the ith age determination of the jth fish

$$(1) \quad \text{Let } X_j = \frac{1}{R}\sum_{i=1}^{R} X_{ij}$$

(X_j is the average age calculated for the jth fish).

Then

$$(2) \quad \frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij} - X_j|}{X_j}$$

is the average error in aging the jth fish, as a fraction of the average of the age estimates. Multiplied by 100 it becomes the average percentage error for the jth fish

and

$$(3) \quad \frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij} - X_j|}{X_j}\right]$$

is the index of average error and multiplied by 100 it becomes the index of average percent error.

The index (average percent error) can be used to compare determinations or readers. The set of determinations for a particular species with a smaller index is more precise, or the reader with a smaller index for several sets of determinations for one or more species would be judged more precise than another reader or another group of readers with a higher index. In all cases, greater precision is achieved as percent error is minimized. For example, the data in Table 1 can be grouped for each reader to give an index of average percent error of 4.4% for reader 1 and 11.6% for reader 2, indicating that reader 1 is more precise than reader 2. The index can also be used to compare the precision of age determinations laboratories. For example, the combined data set of readers 1 and 2 could be compared to another set of readings perhaps from another laboratory. The level of precision could also be compared between structures or species, or both. That is, the set of age determinations in Table 1 may be more precise than a set of otolith age determinations for some other species.

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TABLE 1. Example of a set of walleye pollock (*Theragra chalcogramma*) ages using the fin-ray method.[a]

Fish no.	Reader 1			Reader 2		
	1st	2nd	3rd	1st	2nd	3rd
1	7	6	7	6	5	6
2	7	6	6	6	5	6
3	6	6	5	5	4	6
4	4	4	4	4	4	3
5	4	5	6	6	5	5
6	3	3	3	3	3	2
7	5	5	5	5	4	5
8	4	4	4	5	4	4
9	4	4	4	4	3	3
10	7	8	7	6	5	7
11	7	7	7	7	5	6
12	3	2	3	3	2	3
13	5	5	5	5	4	4
14	4	4	4	5	2	4
15	6	6	4	6	4	5
16	7	7	7	5	6	5
17	6	6	5	5	5	5
18	7	7	7	7	6	6
19	8	7	7	7	5	5
20	5	5	5	5	4	5

[a]All readings were independent of previous readings and other readers' determinations and several months were allowed between readings. Fish numbers represent the sequence of sampling in the field.

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Increased Toxicity of Ammonia to Rainbow Trout (*Salmo gairdneri*) Resulting from Reduced Concentrations of Dissolved Oxygen

ROBERT V. THURSTON, GLENN R. PHILLIPS,[1] AND ROSEMARIE C. RUSSO

Fisheries Bioassay Laboratory, Montana State University, Bozeman, MT 59717, USA

AND SUSAN M. HINKINS

Department of Mathematics, Montana State University, Bozeman, MT 59717, USA

THURSTON, R. V., G. R. PHILLIPS, R. C. RUSSO, AND S. M. HINKINS. 1981. Increased toxicity of ammonia to rainbow trout (*Salmo gairdneri*) resulting from reduced concentrations of dissolved oxygen. Can. J. Fish. Aquat. Sci. **38: 983–988.**

The median lethal concentration (LC50) of aqueous ammonia at reduced dissolved oxygen (D.O.) concentrations was tested in acute toxicity tests with rainbow trout (*Salmo gairdneri*) fingerlings. Fifteen 96-h flow-through tests were conducted over the D.O. range 2.6–8.6 mg/L, the former concentration being the lowest at which control fish survived. There was a positive linear correlation between LC50 (milligrams per litre un-ionized ammonia) and D.O. over the entire D.O. range tested; ammonia toxicity increased as D.O. decreased. Ammonia LC50 values were also computed for 12, 24, 48, and 72 h; the correlation with D.O. was greater the shorter the time period.

[1]Present address: Cooperative Fisheries Research Unit, Montana State University, Bozeman, MT 59717, USA.

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Nous avons déterminé, au cours d'essais de toxicité aiguë avec fingerlings de truite arc-en-ciel (*Salmo gairdneri*), la concentration létale médiane (CL50) de l'ammoniac en solution aqueuse à des concentrations réduites d'oxygène dissous (O.D.). Quinze essais de 96 h à débit continu ont été effectués à des concentrations de O.D. variant de 2,6 à 8,6 mg/L, la première étant la plus faible à laquelle les poissons témoins purent survivre. Il y a corrélation linéaire positive entre la CL50 (mg/L d'ammoniac non ionisé) et O.D. sur toute la gamme de ce dernier testée; la toxicité de l'ammoniac augmente à mesure que O.D. diminue. Nous avons également calculé la CL50 de l'ammoniac après 12, 24, 48 et 72 h; plus la période est courte et plus forte est la corrélation avec O.D.

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AMMONIA is a common component in the discharges from sewage treatment plants and many industrial processes. Reduced dissolved oxygen (D.O.) concentrations are often associated with ammonia effluents because of other characteristics of ammonia-containing wastes, e.g. high chemical and biochemical oxygen demands and elevated temperature. The interactive effect of ammonia and D.O. is, therefore, an important consideration with respect to the toxicity of ammonia to aquatic life.

Several researchers have reported that reduced D.O. concentration increases the toxicity of ammonia to fishes (Wuhrmann 1952; Wuhrmann and Woker 1953; Allan 1955; Downing and Merkens 1955; Herbert 1956; Merkens and Downing 1957; Lloyd 1961; Danecker 1964; Vámos and Tasnádi 1967; Seleši and Vamoš 1976; Alabaster et al. 1979) Results reported are that a decrease in D.O. concentration (generally 30–50% below saturation) increases the toxicity of ammonia, that at a given ammonia concentration the degree of response is correlated to D.O. concentration, that rainbow trout (*Salmo gairdneri*) are more sensitive to the combined effect of reduced D.O. and ammonia than were the other (nonsalmonid) fishes tested, and that salinity reduces the acute toxicity of ammonia to rainbow trout under reduced D.O. conditions. It has also been reported that the effect of oxygen in increasing the survival time of rainbow trout fingerlings is greater at low concentrations of ammonia than at high (Downing and Merkens 1955; Merkens and Downing 1957).

In the present study we conducted a series of fifteen 96-h flow-through toxicity tests to examine the effects of reduced D.O. concentrations on the acute toxicity of ammonia to rainbow trout fingerlings. Ammonia toxicity was measured at different concentrations of D.O. over the range 2.6–8.6 mg/L, and at different time periods. Such an approach has not been used in previously reported studies. The data were analyzed to examine (1) the effect of different concentrations of D.O. on the acute toxicity of ammonia, (2) whether such an effect varied with time, and (3) whether such an effect was greater at low than at high ammonia concentrations.

Methods — Test fish were Shasta–Manchester hybrid rainbow trout from a single lot of fish obtained from the Bozeman (Montana) Fish Cultural Development Center (FCDC), U.S. Fish & Wildlife Service (F&WS). The tests were conducted over a 2-mo period when the fish were 4 to 6 mo old. Prior to testing, the fish were reared in water (Table 1) from a ground spring located at FCDC; this same water was used for the test dilution water. During rearing, the fish were fed a F&WS hatchery production diet (Rangen Inc., Buhl, ID); they were not fed 3 d prior to, or during, test. Two tests were conducted at the normal D.O. concentration of the ground spring water and 13 tests were conducted at reduced D.O. concentrations. Reduced D.O. concentrations were achieved by mixing water taken directly from the ground spring source with water from the ground spring which had been passed through a degassing system similar to that described by Mount (1964).

Six tanks (water volume 60 L each) were used for each test, five test tanks and one control tank. Test water was delivered to the tanks by either of two methods. In the two tests at the highest D.O. concentration (8.6 mg/L), toxicant (reagent grade ammonium chloride) solution was delivered to the tanks at a flow rate of 500 mL at 2- to 3-min intervals from proportional diluters having the basic design of Mount and Brungs (1967); water replacement time in the tanks was ~5 h and full test toxicant concentration in each tank was effectively reached within 18 h. In tests at reduced oxygen concentrations, deoxygenated dilution water was delivered into the tanks

TABLE 1. Chemical characteristics of the dilution water used in bioassays (all values are milligrams per litre unless noted otherwise).

Al	<1	Ni	<0.005
As	0.0012	Pb	<0.015
Ca	52	Se	0.00085
Cd	<0.005	Zn	0.01
Cr	<0.005	Cl^-	0.16
Cu	0.007	F^-	0.35
Fe	0.004	PO_4^{3-}	0.05
Hg	<0.00005	SO_4^{2-}	17
K	0.82	Total organic carbon	3.3
Mg	17	SEC, μS 25°C	340
Mn	0.002	Turbidity, NTU	1.6
Na	2.5		

TABLE 2. Toxicity of ammonia to rainbow trout under different conditions of dissolved oxygen[a] (10 fish per tank in all tests).

Test No.	Mean fish size		D.O. (mg/L) (range)	pH (range)	Temp. (°C) (range)	96-h LC50 (95% C.I.)	
	Wt (g)	Length (cm)				mg NH_3–N/L	mg NH_3/L
397	1.7	5.7	8.61	7.79	12.4	42.0	0.696
			(8.40–8.95)	(7.75–7.85)	(12.1–12.7)	—[b]	—
404	2.3	6.1	8.58	7.80	12.4	47.9	0.812
			(8.40–8.90)	(7.77–7.87)	(11.8–13.1)	(43.3–53.0)	(0.734–0.899)
436	5.7	8.4	7.7	7.80	13.3	42.0	0.763
			(7.5–7.9)	(7.76–7.86)	(12.9–13.8)	(37.9–46.6)	(0.689–0.846)
444	4.0	7.2	7.57	7.83	12.8	36.5	0.683
			(7.40–7.75)	(7.76–7.96)	(12.6–13.0)	(31.2–42.7)	(0.584–0.800)
437	5.7	8.0	7.37	7.79	12.9	40.9	0.704
			(7.10–7.65)	(7.75–7.85)	(12.7–13.2)	(36.9–45.3)	(0.636–0.780)
430	5.7	8.0	6.60	7.75	12.5	37.0	0.564
			(6.50–6.90)	(7.70–7.82)	(12.3–12.7)	(33.4–40.9)	(0.510–0.624)
426	5.0	7.6	6.57	7.76	12.5	39.1	0.610
			(6.35–6.75)	(7.73–7.79)	(12.2–12.9)	(35.6–42.9)	(0.555–0.670)
420	4.6	7.3	5.66	7.75	12.7	32.1	0.497
			(5.50–5.90)	(7.72–7.79)	(12.4–13.1)	(30.2–34.0)	(0.468–0.527)
414	3.2	6.5	5.47	7.75	13.0	40.6	0.643
			(5.10–5.80)	(7.71–7.79)	(12.6–13.2)	—	—
398	1.7	5.6	4.40	7.76	12.5	33.1	0.517
			(4.25–4.60)	(7.70–7.83)	(12.4–12.7)	(30.4–36.1)	(0.474–0.564)
405	2.3	6.2	4.37	7.78	12.4	33.4	0.541
			(4.25–4.55)	(7.74–7.84)	(12.0–13.0)	(31.8–35.0)	(0.516–0.567)
450	9.4	9.6	3.56	7.87	12.6	23.9	0.482
			(3.30–3.95)	(7.80–7.95)	(12.5–12.7)	(21.6–26.6)	(0.436–0.536)
452	10.1	9.7	3.21	7.79	12.6	23.7	0.399
			(2.65–3.45)	(7.75–7.86)	(12.4–12.9)	—	—
464	8.8	9.1	2.65	7.92	12.8	14.4	0.330
			(2.50–2.85)	(7.89–7.96)	(12.7–13.0)	(11.8–17.5)	(0.271–0.401)
457	8.2	8.7	2.64	7.89	12.5	15.1	0.316
			(2.40–2.95)	(7.85–7.95)	(12.2–12.9)	(13.3–17.1)	(0.280–0.358)

[a]Averages and range of values in all tanks for all tests for other measured water chemistry variables (in milligrams per litre) were alkalinity (as $CaCO_3$), 172 (169–176); hardness (as $CaCO_3$), 205 (203–210); NO_2–N, 0.00 (0.00–0.01); and NO_3–N, 0.14 (0.06–0.19).

[b]Confidence intervals not calculable from statistical method used.

under constant pressure through flowmeters (Dwyer Instrument Corp.) at the rate of 100 mL/min. In these tests, toxicant solution was premixed into the dilution water at different rates for each tank by means of fixed-speed metering pumps (Barnant Corp.) and water replacement time in the tanks was ~10 h; full test toxicant concentration in each tank was effectively reached within 36 h.

Fish for a given test were selected at random 2–2.5 d prior to the start of the test, weighed collectively, and distributed (10 per tank) at random among the tanks. The D.O. concentration of the tank water into which the fish were placed was 8–9 mg/L. This water was then gradually replaced by water at the test D.O. concentration over the next 18–36 h; test D.O. concentration was effectively reached 0.5–1 d before introduction of toxicant. D.O. and flow rates for each tank were measured at least twice each day thereafter and flow rates were adjusted if needed. The lowest D.O. test completed was 2.6 mg/L, this being the lowest concentration at which 90% or more of the control fish survived. Measurements of fish for total length were taken as dead fish were removed from the tanks during each test or at the conclusion of each test.

Chemical analyses were performed on water in the test tanks using the following methods. Total ammonia–nitrogen (NH_3–N) was determined three or four times in each tank for each test (beginning on day 2) using the nesslerization method from APHA et al. (1976). Un-ionized ammonia (NH_3) concentrations were calculated from total ammonia concentrations in the individual test tanks using the formulas of Emerson et al. (1975) and the table of Thurston et al. (1979). Oxygen was measured using a Yellow Springs Instrument model 54 D.O. meter, temperature using a calibrated mercury thermometer, and pH with a Beckman Phasar-I digital meter. These measurements were taken each time total ammonia was measured. Average pH values were obtained by use of a computer program which converted each measurement to the hydrogen ion concentration, averaged these, then reconverted the average to pH units. Alkalinity, hardness, nitrate–nitrogen (NO_3–N), and nitrite–nitrogen (NO_2–N) were determined at least once in each tank for each test according to standard analytical procedures (APHA et al. 1976; U.S. EPA 1974). Colorimetric measurements were made using a Varian 635 ultraviolet–visible spectrophotometer.

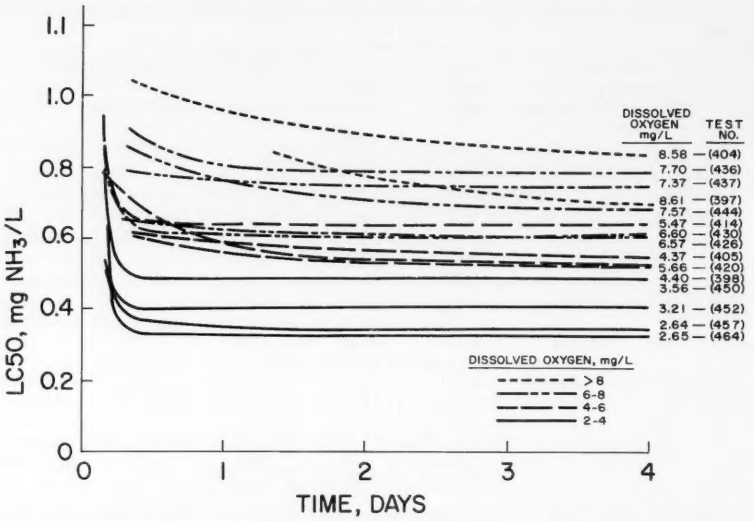

FIG. 1. Ammonia toxicity curves for 96-h bioassays on rainbow trout at different concentrations of dissolved oxygen.

Mortality observations were made at 4- to 8-h intervals during the first 24 h of each test and at 6- to 12-h intervals during the next 72 h. The median lethal concentrations (LC50) and their 95% confidence intervals were calculated for each test from the average NH_3–N and NH_3 values for each tank using the trimmed Spearman–Karber method (Hamilton et al. 1977). Toxicity curves were computer-generated using LC50 and time values for each time of mortality observation ($\geq$12 per test).

The 96-h LC50 (as milligrams NH_3 per litre) and D.O. (milligrams per litre) data were subjected to statistical correlation calculations. LC50 and ln (LC50) were both examined for possible correlations with D.O. concentration (including ln (D.O.) and $(D.O.)^2$). Because weight and length are always highly correlated (estimated correlation >0.95), and because weight is usually more highly correlated with LC50 than is length, we considered only weight as a possible covariable and calculated partial correlations for the 96-h LC50/D.O. data, holding fish weight constant. The correlations of D.O. vs. LC50 for the time periods of 12, 24, 48, 72, and 96 h were also compared to examine whether the effect of reduced oxygen concentration on ammonia toxicity was affected by duration of exposure. Finally, we examined our data to determine whether there was any evidence in our experiments of a greater effect of D.O. on survival time at low than at high concentrations of NH_3. For each test (each different D.O. concentration), the number of mortalities at 96 h for each of the five ammonia test tank concentrations was tabulated. The following linear relationship was assumed:

$$p = \beta_0 + \beta_1 \ln (NH_3) + \beta_2 \ln (D.O.) + \beta_{12} \ln (NH_3) \ln (D.O.)$$

where p = proportion mortality at 96 h, NH_3 = concentration of un-ionized ammonia in milligrams per litre, and D.O. = concentration of dissolved oxygen in milligrams per litre. The term $\beta_{12} \ln (NH_3) \ln (D.O.)$ is the interaction effect between NH_3 and D.O.

The hypothesis that D.O. concentration has a greater effect at low NH_3 concentration than at high can be restated as the hypothesis that β_{12}, the coefficient on the interaction term, is strictly positive; one expects β_2 to be negative. Therefore, the procedure used was to run a weighted linear regression of p on ln (NH_3), ln (D.O.), and the product ln (NH_3) ln (D.O.), test whether the coefficient β_{12} is significantly different from zero, and, if so, if it is positive. For completeness two other models to equalize variance were used (Snedecor and Cochran 1967); one of these involved arcsin transformation and the other logit transformation.

Results and discussion — Ninety-six-hour LC50 values (with 95% confidence intervals) under different conditions of D.O. are reported (Table 2) in terms of both NH_3–N and NH_3. Fish size and water measurements for D.O., pH, and temperature for each test are also reported (Table 2), as are summaries of the measured water chemistry variables considered not to have affected test results. There was one control mortality during one of the tests at the lowest D.O. level tested (test 464); there were no control mortalities in the other tests. Toxicity curves in terms of NH_3 are presented in Fig. 1. Most of the toxicity curves plateau by 4 d, giving asymptotic LC50 values ranging from 0.32 to 0.81 mg NH_3 per litre.

There was a strong positive correlation between D.O. (milligrams per litre) and LC50 (milligrams NH_3 per litre); as D.O. concentration decreased, NH_3 toxicity increased. The linear relationship between 96-h LC50 and D.O. concentration is illustrated in Fig. 2. The estimated correlation coefficient is 0.9346 (P = 0.00001; the estimated regression line

is LC50 = 0.1903 + 0.06712 (D.O.)). The highest estimated correlation of those calculated was between ln (LC50) and ln (D.O.) and equalled 0.9471 ($P = 0.00001$). The estimated partial correlation between ln (LC50) and ln (D.O.) holding weight fixed was 0.9064 ($P < 0.01$).

Analysis of the data to compare the D.O. vs. LC50 correlations at 12, 24, 48, 72, and 96 h showed a very clear trend (Fig. 3); the shorter the time period, the more pronounced the positive relationship between LC50 and D.O. concentration ($P < 0.0001$). This trend suggests at least two possibilities: either individual fish that require higher oxygen concentrations succumb early in the tests and/or those fish that do survive become increasingly acclimated to the ammonia and oxygen test conditions as time progresses.

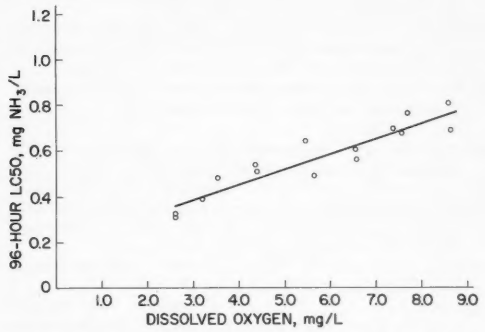

FIG. 2. Effect of dissolved oxygen on the acute toxicity of ammonia to rainbow trout: 96-h LC50 vs. D.O.

To test the hypothesis that the magnitude of the effect of reduced D.O. on ammonia toxicity is greater at low than at high concentrations of NH_3, we used both complete and truncated data sets. Analyzing the complete data set, using weighted linear regression, gives the following estimated model:

$$p = 1.86 + 0.734 \ln (NH_3) - 0.493 \ln (D.O.) + 0.031 \ln (NH_3) \ln (D.O.).$$

The estimated coefficient of the interaction term is then $\hat{\beta}_{12} = 0.031$. The coefficients on ln (NH_3) and ln (D.O.) are both significant (with P values of 0.000 and 0.002, respectively) but the coefficient for the interaction is not significantly different from zero ($P = 0.818$). Therefore, there is no evidence of any interaction between NH_3 concentration and D.O.

The truncated set consisted of only those treatments in which mortality was greater than 0% and less than 100%. The reasoning behind this truncation is that if no fish die at a low concentration of NH_3 coupled with a low level of D.O., no fish should die as the level of D.O. increases. Similarly, if all fish die at a high concentration of NH_3 coupled with a high level of D.O., there should still be 100% mortality as the level of D.O. decreases. When the truncated data set was used to run a weighted regression of p vs. ln (NH_3), ln (D.O.), and the product, all three coefficients were significant; there was a significant positive interaction ($P = 0.006$) and the hypothesis is accepted. This is a contradiction of the previous result. The analyses using arcsin transformation and logit transformation also yielded results that were not conclusive.

The discrepancy in results between using a complete data set and a truncated data set may be due to the inadequacy of

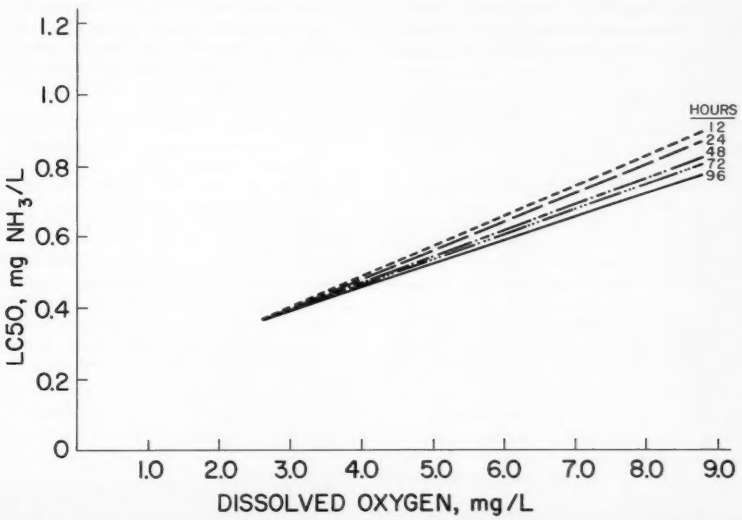

FIG. 3. Effect of dissolved oxygen on the acute toxicity of ammonia to rainbow trout: LC50 vs. D.O. at five time intervals.

the model to describe mortality behavior at the extreme values of p (0 or 1). The model may be more appropriate for the truncated data set, because this set was limited to those data covering the range wherein a change in concentration of D.O. and/or NH_3 had an effect on mortality. It would be unwise to draw any conclusions about interaction between NH_3 concentration and D.O. from only these data; future research should further address the question of interaction and a more complete model should be considered, one which might better handle the method of determining the region of D.O. and NH_3 values of interest.

Independent of conclusions from the preceding data analysis, the 96-h LC50 values are more often reached earlier in the tests at low D.O. concentrations than at high. This point is illustrated both by the 96-h toxicity curves (Fig. 1) and by the differences in slope of the regression lines (Fig. 3); the longer the time period, the less the slope.

The U.S. Environmental Protection Agency (1977) has recommended a minimum concentration of 5.0 mg D.O. per litre to maintain good freshwater fish populations. Our results show that any reduction in D.O., below the highest tested, reduced the tolerance of rainbow trout fingerlings to acutely toxic concentrations of ammonia; the estimated tolerance at 5.0 mg D.O. per litre is 30% less than that at 8.5 mg D.O. per litre. We recommend that water quality standards for D.O. in cold-water fish environments take into consideration background concentrations of ammonia which may be present and the likelihood of temporary increases in those concentrations.

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Abundance, Life History, and Growth of Introduced Populations of the opossum shrimp (*Mysis relicta*) in Subalpine California Lakes

MARK D. MORGAN[1]
Division of Environmental Studies, University of California — Davis, Davis, CA 95616, USA

MORGAN, M. D. 1981. Abundance, life history, and growth of introduced populations of the opossum shrimp (*Mysis relicta*) in subalpine California lakes. Can. J. Fish. Aquat. Sci. 38: 989–993.

The opossum shrimp, *Mysis relicta*, was introduced into Donner Lake and Fallen Leaf Lake, California, in 1965. Samples from both lakes in August 1978 and June 1979 and from Donner Lake in November 1980, reveal that the mysids are established throughout each lake. In Donner Lake, the estimated overall lakewide density was 238/m^2 in August 1978, 123/m^2 in June 1979, and 108/m^2 in November 1980. In Fallen Leaf Lake, the overall lakewide density was 114/m^2 in August 1978 and 103/m^2 in June 1979. Mysids in both populations lived for 2 yr and reproduced each spring at 1 and 2 yr of age. Growth was fastest in the spring and summer and slowest in the fall and winter. Previously published data suggest that mysid life history traits can be predicted based on the productivity of the lake. This study supports this view. When productivity is relatively high, the life cycle lasts from 1 to 2 yr, but when productivity is low, it may take up to 4 yr.

Key words: *Mysis relicta*, life history, species introduction, fisheries management, Fallen Leaf Lake, Donner Lake, Lake Tahoe

MORGAN, M. D. 1981. Abundance, life history, and growth of introduced populations of the opossum shrimp (*Mysis relicta*) in subalpine California lakes. Can. J. Fish. Aquat. Sci. 38: 989–993.

On introduisait en 1965 des mysides, *Mysis relicta*, dans les lacs Donner et Fallen Leaf de la Californie. À en juger par des échantillons recueillis dans les deux lacs en août 1978 et juin 1979, et dans le lac Donner en novembre 1980, l'espèce s'était établie à la grandeur de chaque lac. Dans le lac Donner, la densité pour l'ensemble du lac était estimée à 238/m^2 en août 1978, à 123/m^2 en juin 1979 et à 108/m^2 en novembre 1980. Dans le lac Fallen Leaf, la densité pour l'ensemble du lac était de 114/m^2 en août 1978 et de 103/m^2 en juin 1979. Les sujets des deux populations vécurent 2 ans et se reproduisirent chaque printemps aux âges 1 et 2. La croissance est la plus rapide au printemps et en été, et la plus lente en automne et en hiver. Des données déjà publiées suggèrent que les caractéristiques du cycle biologique des mysides peuvent être prédites à partir de la productivité du lac. La présente étude confirme cette hypothèse. Quand la productivité est relativement forte, le cycle biologique dure de 1 à 2 ans, mais quand elle est faible, le cycle peut durer jusqu'à 4 ans.

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THE opossum shrimp, *Mysis relicta*, is central to the dynamics of many sport and commercial fisheries species, both in lakes where it occurs naturally, and where it has been introduced (Hacker 1956; Wells and Beeton 1963; Northcote 1972; Morgan et al. 1978; Olsen 1980). As a result, there is a great demand for detailed information on the various aspects of its life history. Numerous studies have indicated that the life history characteristics of *M. relicta* differ greatly from one environment to the next (cf. Lasenby and Langford 1972; Morgan 1980). Therefore, life history information must be obtained on a population by population basis, which traditionally is a costly and time-consuming process. Recently, however, a means of predicting the life history traits of *M. relicta* based primarily on the productivity of the environment has been proposed (Morgan 1980).

The purpose of this note is to provide basic life history information on two introduced populations of *M. relicta* in Donner Lake and Fallen Leaf Lake, California, and to determine how well these characteristics fit in with those expected (see Morgan 1980). Analysis of the Donner and Fallen Leaf Lake data has been fascilitated by comparison with more detailed and previously published information (Morgan 1980) from Lake Tahoe and Emerald Bay, an isolated, for *Mysis*, embayment of Lake Tahoe.

Study area — Donner and Fallen Leaf Lake are located near Lake Tahoe on the eastern slope of the Sierra Nevada at elevations of 1809 and 1939 m, respectively (Fig. 1). Fallen

[1]Present address: Department of Biology, Rutgers University, Camden, NJ 08102, USA.

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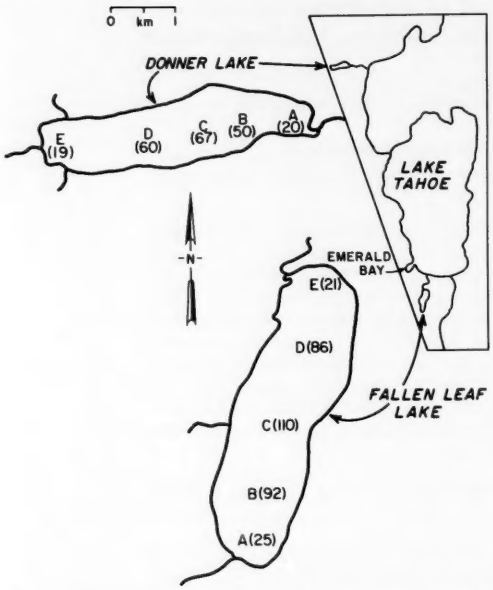

FIG. 1. Donner Lake and Fallen Leaf Lake, California, with location relative to Lake Tahoe and Emerald Bay, and the position and depths (in parentheses) of sampling stations.

Leaf Lake is slightly larger and deeper (5.6 km^2, z_{max} = 110 m) than Donner Lake (3.8 km^2, z_{max} = 67 m) and both are of glacial origin, with characteristically steep sloping sides. *Mysis relicta* was introduced into Donner and Fallen Leaf Lake in 1965. The Lake Tahoe and Emerald Bay introductions occurred from 1963 to 1965 (Hansen 1966).

The limnological characteristics of Donner Lake, Fallen Leaf Lake, Lake Tahoe, and Emerald Bay are generally similar (Dong 1975; Goldman and Rundquist 1977; C. R. Goldman unpublished data). There are no major differences in light, temperature, nutrients, and biota among these systems. Minor hypolimnial oxygen depletion occurs during summer stratification in Donner Lake, but does not appear to have any significant biological effects (Goldman and Rundquist 1977). Measurement of lake productivity based on ^{14}C uptake and secchi depths for each lake and Emerald Bay was obtained in August 1978 (Table 1). This represents the only occasion when the productivity of all four bodies of water was measured at the same time. Both the secchi depth and carbon assimilation data indicate that Donner Lake, Fallen Leaf Lake, and Emerald Bay are similar in overall productivity while Lake Tahoe is much lower. Although these data cannot provide a rigorous comparison of the relative productivity of these systems, they do confirm previous production measurements (Morgan et al. 1981) and general experience with these systems.

Materials and methods — Mysis relicta was sampled in August 1978, June 1979, and November 1980 from Donner Lake and August 1978 and June 1979 from Fallen Leaf Lake

TABLE. 1. Comparison of secchi depths and primary productivity (mgC · m^{-2} · d^{-1}) from Emerald Bay, Donner Lake, Fallen Leaf Lake, and Lake Tahoe in August 1978.

Lake	Secchi depth (m)	Productivity[a] (mgC · m^{-2} · d^{-1})	Date
Emerald Bay	15	6.3	Aug. 31, 1978
Donner Lake	12	5.3	Aug. 30, 1978
Fallen Leaf Lake	14	5.9	Aug. 17, 1978
Lake Tahoe	26	1.9	Aug. 28, 1978

[a]Based on ^{14}C uptake in light and dark bottles throughout the euphotic zone. See Morgan et al. (1981) and Goldman (1974) for further details.

by night vertical net tows taken from the bottom to the surface with a $\frac{2}{3}$-m-diameter plankton net (0.5-mm mesh). Towing speed was maintained at about 0.5 m/s. One or two tows were taken at each of five stations located along a longitudinal transect in both lakes, except for June in Fallen Leaf Lake when only two stations were sampled due to bad weather (Fig. 1). Details of *Mysis* sampling in Lake Tahoe and Emerald Bay have been previously reported (Morgan 1980). Samples were preserved in 10% Formalin.

All individuals in a sample were counted after which replicate samples were pooled. If necessary, a Folsom plankton splitter was used to reduce a pooled sampled to form 100 to 200 individuals for size frequency analysis and sex determination. Individuals were measured to the nearest 0.5 mm from the tip of the rostrum to the end of the telson using a dissecting microscope. Mysids larger than 12 mm were sexed based on the presence of enlarged 4th pleopods in the males and rudimentary oöstigite plates on the last thoracic segments in the females. Mature males and gravid females were classified according to Morgan (1980). Average lakewide size frequency distributions on each date were obtained by averaging the size frequency distributions in proportion to the densities obtained at each station.

Results and discussion — Mysis relicta is clearly established throughout both Donner and Fallen Leaf lakes. Large numbers of individuals were collected from both lakes at all stations in both August 1978 and June 1979, and in Donner Lake on November 3 1980 (Table 2). Average lakewide mysid densities in Donner Lake were 238/m^2 in August, 123/m^2 in June, and 108/m^2 in November. In Fallen Leaf Lake, the average lakewide density was 114/m^2 in August 1978. Since bad weather prevented sampling at three stations in June 1979, no reliable estimate of the lakewide density could be made, although the average density at the two stations sampled was 103/m^3.

The life history characteristics of introduced populations of *M. relicta* in nearby Lake Tahoe and Emerald Bay have been successfully worked out by analysis of changes in the population size structure over time (Morgan 1980). Similarity in the growth patterns of successive cohorts at corresponding times was a major factor which made these analyses possible. For example, the size frequency distributions of *Mysis* in Emerald Bay from June to October 1977 are virtually identical with the distributions obtained from this population during the same period in 1979 (Fig. 2). The following analysis of the life history of mysids from Donner and Fallen Leaf Lake

TABLE. 2. Average densities (no./m^2) of *Mysis relicta* at each station and lakewide average densities in Donner Lake on August 30, 1978, June 15, 1979, and November 3, 1980 and in Fallen Leaf Lake on August 17, 1978 and June 13, 1979. NS = no sample.

	Donner Lake				Fallen Leaf Lake		
Station	z (m)	Aug. 30, 1978	June 15, 1979	Nov. 3, 1980	z (m)	Aug. 17, 1978	June 13, 1979
A	20	333	24	144	25	169	71
B	50	199	133	99	92	223	134
C	67	184	168	74	110	60	NS
D	60	186	252	102	86	77	NS
E	19	287	95	119	21	43	NS
Average lakewide density		238	123	108		114	103

depends upon the assumption that growth patterns of successive cohorts are similar. Acceptance of this assumption, the validity of which will be discussed below, leads to two conclusions. First, any given cohort's life history can be deduced by following the development of younger or older cohorts, and second, the year in which size frequency distributions are obtained is unimportant for working out growth patterns, only the time of year matters. Therefore, to aid in the visualization of the life history of these populations, the June 1978 size frequency distributions are treated as if they are identical with the observed distributions from June 1979 (Fig. 3).

There is little difference in the Donner and Fallen Leaf Lake size frequency distributions for corresponding periods (Fig. 3). Consequently, the life history traits of these populations appear very similar and will be discussed together. There is also remarkably little difference between the Donner and Fallen Leaf Lake distributions and the Emerald Bay distributions (Fig. 2) at similar times of the year. Thus, the more detailed information on the life history of the Emerald Bay mysids (Morgan 1980) can serve as a model to outline the life history of the Donner and Fallen Leaf Lake mysids.

Young in Emerald Bay are released at 4 mm in length only from February to April and by June attain a mean length of 8.5 mm (Fig. 2; Morgan 1980). A similar production of young in Donner and Fallen Leaf Lake is indicated by the presence of a distinct cohort with a mean length of about 6.5 mm in June (Fig. 3). The smaller size of this cohort compared with the Emerald Bay cohort suggests young are released slightly later in the spring. This cohort can be easily followed through August when it attains a mean length of 10.5 mm. In November, this cohort begins to merge with the older cohort and becomes reproductive. The males and females range in size from 9.5 to 14 mm and 9 to 15 mm, respectively, but mature males and gravid females are present only at the upper ends of these ranges. In Emerald Bay, ~75% of the males and 40% of the females become reproductive their first year. Although the November samples were taken at the beginning of the reproductive period, the percentage of individuals which will eventually become reproductive appears similar to that for Emerald Bay. After releasing young in the spring, this cohort, now in its second year, attains a mean length of 15.5 mm in June and grows to 16.5 mm in August. It becomes reproductive again in November with mature males and females ranging in size from 15.5 to 16.5 and 15.0 to 18.5 mm, respectively. There is no overlap in the size of males from the first and second-year cohorts and only minimal overlap among females. As indicated from the data on mysids in Emerald Bay, the 2-yr-old females will become gravid later in the fall and release young the following spring. Thus, mysids in Donner and Fallen Leaf Lake exhibit life history patterns remarkably similar to those from Emerald Bay, with individuals living for up to 2 yr and reproduction occuring by both 1- and 2-yr-old individuals.

The preceeding analysis depended heavily on the assumption that year-to-year variation in life history and growth patterns did not occur. As discussed earlier, the extensive data from Emerald Bay supports this assumption. However, its applicability to mysids from Donner and Fallen Leaf Lake cannot be directly assessed since samples from corresponding periods of different years were never taken. Nevertheless, there are certain internal consistencies which suggest that this assumption is valid. First, the November 1980 size frequency distributions fits in perfectly with the pattern established in August 1978, even though none of the members of the 1978 population were still present in 1980. Second, there is great similarity in the June 15, 1979 and November 3, 1980 size frequency distributions from Donner Lake to the June 12, 1977 and October 31, 1977 distributions from Emerald Bay. The possibility that these similarities result from coincidence must be considered slight. Thus, even in two different bodies of water, separated in time by 3 years, the progression of life history events remained virtually identical. Consequently, constancy in population development appears to be a real attribute of *M. relicta* in these lakes.

The mean size of each cohort identified in the preceeding discussion from Donner and Fallen Leaf Lake is plotted against time to yield a growth curve (Fig. 4). Growth data determined in a similar manner for mysids in Emerald Bay and Lake Tahoe are also plotted for comparison. A curve (broken line) identical with that fitted to the Emerald Bay growth data has been applied to the Donner and Fallen Leaf Lake data with a 1-mo delay. It is clear from the graph that the fit is very good, suggesting that there are no significant differences in the growth rates among the three populations. The lag in growth can be accounted for by slightly delayed release of young as discussed earlier.

Growth in Donner Lake, Fallen Leaf Lake, and Emerald Bay is significantly faster than in Lake Tahoe where it takes

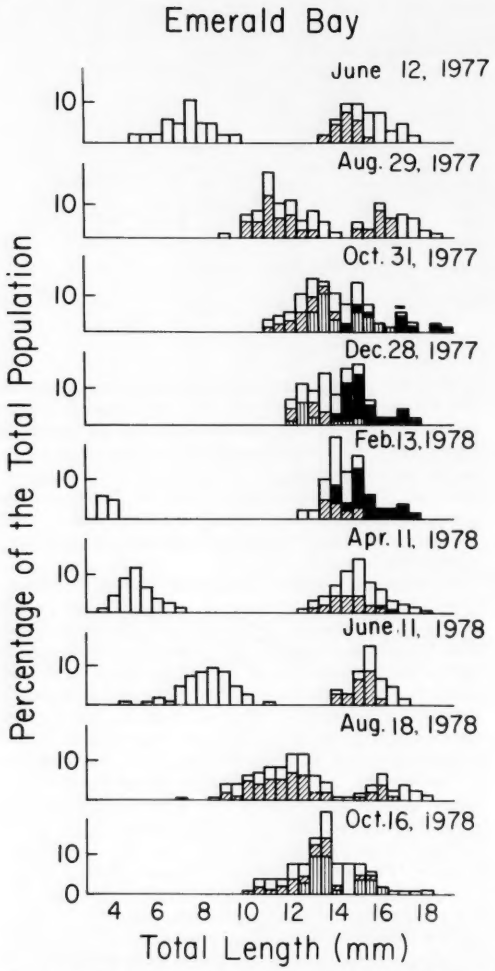

FIG. 2. Size frequency distributions of *Mysis relicta* in Emerald Bay. Open bars represent females or sexually undifferentiated individuals. Solid bars indicate gravid females. Diagonally hatched bars represent males and vertically hatched bars indicate mature males. From Morgan (1980). (Reprinted by permission of the Ecological Society of America. In *Ecology* 61: 553, Fig. 2, 1980)

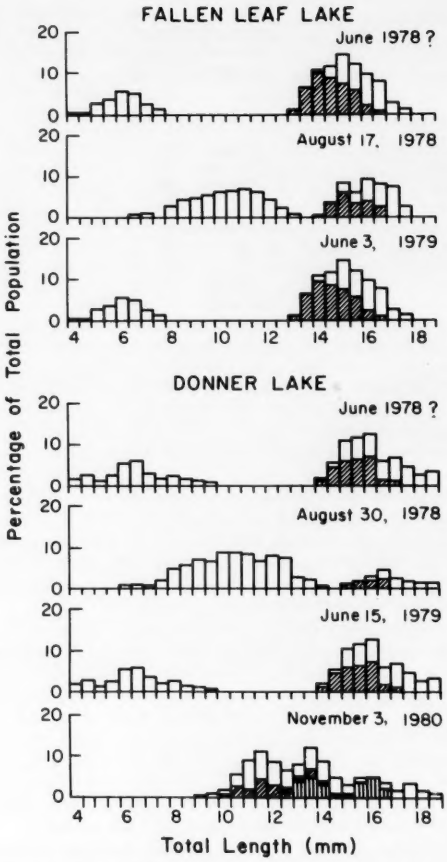

FIG. 3. Size frequency distributions of *Mysis relicta* in Donner Lake and Fallen Leaf Lake over the sampling period. Symbols are the same as in Fig. 2. For each lake, the June 1979 size frequency distributions is replotted assuming it would be identical with the June 1978 distribution.

4 years to reach the size attained by the other mysid populations in just 2 years (Morgan 1980). Even so, the overall growth patterns of all the populations are similar (Fig. 4). The growth rate increases rapidly through the first summer of the cohorts life, slows in the fall and winter, increases again the second spring and summer, and finally slows (and stops in the case of Emerald Bay, Donner and Fallen Leaf Lake) in the fall and winter of the 2nd year (Fig. 4). The slower growth in the fall and winter can be most easily associated with the general decrease in overall lake productivity characteristic of temperate lakes at these times of the year. It should be noted, however, that a large portion of the Emerald Bay cohort, and by inference, the Donner and Fallen Leaf Lake cohorts, were reproductive in their 1st year and that the entire cohort was reproductive in its 2nd year. Thus, a significant portion of the reduced growth may simply represent a shift of energy from growth to reproduction. This may explain the greater rate of decline in growth observed in Emerald Bay, Donner Lake, and Fallen Leaf Lake compared with Lake Tahoe since the Lake Tahoe cohort was not reproductive at all its first 2 years (Morgan 1980).

The life history and growth patterns of *M. relicta* have now been examined in a number of different environments and are becoming quite well understood. It has been suggested that the major aspects of these traits (length of life, continuous vs. noncontinuous reproduction, and age and size at maturity) can

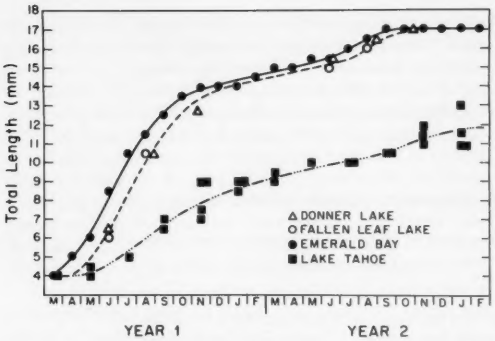

FIG. 4. Growth of *Mysis relicta* in Donner Lake, Fallen Leaf Lake, Lake Tahoe, and Emerald Bay. Points obtained by plotting the mean size of an individual cohort against time from when it was released. Curves are drawn by inspection. The Donner Lake and Fallen Leaf Lake growth curve is drawn identical with the Emerald Bay curve assuming a 1-mo. lag.

be predicted based on the productivity characteristics of the environment (Morgan 1980). The productivity of Donner and Fallen Leaf Lake is similar to Emerald Bay (Table 1) as are the life history characteristics of the mysid populations. On the other hand, Lake Tahoe is much less productive and the mysid life history characteristics are accordingly much different. Thus, the overall importance of productivity is supported by these data.

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Absorption of Selected Antimicrobic Drugs from Water by Channel Catfish, *Ictalurus punctatus*[1]

KENNETH E. NUSBAUM AND EMMETT B. SHOTTS JR.

Department of Medical Microbiology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA

NUSBAUM, K. E., AND E. B. SHOTTS. 1981. Absorption of selected antimicrobic drugs from water by channel catfish, *Ictalurus punctatus*. Can. J. Fish Aquat. Sci. 38: 993–996.

Although anitmicrobial drugs are frequently added to water to treat bacterial diseases of fish, their absorption is largely unquantified. Channel catfish weighing 3–5 g each were exposed to varying concentrations (4 mg/L to 32 mg/L) of chloramphenicol, erythromycin,

[1]Portions of this paper are from K. Nusbaum's M.S. thesis.

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furpyrinol, and oxytetracycline, respectively. Antibiotic levels measured after 5 h utilizing *Bacillus subtilus* spore germination inhibition showed that furpyrinol and oxytetracycline were absorbed by the fish in proportion to the antimicrobial concentration in the water. Minimum inhibitory concentrations can be attained in the fish with practical concentrations of these drugs in the water. Erythromycin (carrier-free) was poorly absorbed and chloramphenicol was not absorbed. Significant antibiotic degradation in water occurred in all drugs except furpyrinol.

Key words: fish, antimicrobic, chloramphenicol, erythromycin, furance, oxytetracycline

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Dans le traitement des maladies bactériennes des poissons, on ajoute souvent à l'eau des substances antimicrobiennes, sans qu'on en quantifie précisément l'absorption. Des barbues de rivière pesant de 3 à 5 g chacune furent exposées à des concentrations variables (4 à 32 mg/L) de chloramphénicol, érythromycine, furpyrinol et oxytétracycline, respectivement. Les niveaux d'antibiotique mesurés 5 h après, l'inhibition de la germination de spores de *Bacillus subtilus* servant d'indice, montrent que le furpyrinol et l'oxytétracycline sont absorbés par les poissons dans la même proportion que la concentration de l'antibiotique dans l'eau. On peut obtenir des concentrations inhibitrices minimales chez les poissons en ajoutant à l'eau des concentrations pratiques de ces substances. L'érythromycine (sans porteur) est mal absorbée, alors que le chloramphénicol ne l'est pas du tout. Pour toutes les drogues, sauf le furpyrinol, il y a dégradation marquée de l'action antibiotique dans l'eau.

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EFFICACIOUS antimicrobial therapy for control of bacterial diseases in fish is related to the minimum inhibitory concentration (MIC) of the drug for a given bacterial isolate and the probability of achieving 2–4 times the MIC in the fish. Since diseased fish often do not feed (Amend 1969), and they cannot be expected to absorb significant amounts of antibiotic via the gastrointestinal (GI) tract (Fribourgh et al. 1969). Thus, antimicrobial efficacy must in part be judged on the ability of the drug selected to cross from the water through either the gill epithelium and/or the cutaneous membranes.

The antibiotics selected for these studies traverse mammalian membranes readily. Chloramphenicol is absorbed from the GI tract of vertebrates over a broad pH range (2–9) (Goodman and Gilman 1970) and hence should be useful in the treatment of bacterial disease in fish via water. Erythromycin is absorbed primarily in the alkaline environment of the small intestine of mammals (Goodman and Gilman 1970). It is useful in control of the vertical transmission of *Renibacterium salmoninarum* in egg treatment at spawning (G. W. Klontz, Moscow, ID, personal communication).

Even low concentrations of furpyrinol are easily absorbed from the water by fish and crustaceans without pH dependence (Dainippon Pharmaceutical Ltd., Osaka, Japan). Oxytetracycline is more easily absorbed by animals from a neutral or slightly acid pH (Aronson and Kirk 1974). Absorption of oxytetracycline from the proximal GI tract is incomplete in vertebrates; however, acceptable plasma concentrations of the antibiotic are achieved (Goodman and Gilman 1970). Absorption from the gut and resultant plasma concentrations in fish have been well documented for this compound (Fribourgh et al. 1969).

The purpose of the studies was to determine the amount of each of these antibiotics absorbed from water by fish and to determine if the amount absorbed approached the MIC for several of the more common bacterial pathogens of fish as described by Nusbaum and Shotts (1981).

Methods and Materials — Four channel catfish, *Ictalurus punctatus*, weighing 3–5 g each, were used in each replicate experiment and were held in 8.5 L inert, impervious, plastic buckets containing 3.8 L of aerated water. The water was dechlorinated tap water at 20 ± 1°C, pH 6.7, alkalinity 0, hardness 20 mg/L magnesium and 0.0 mg/L calcium. The water was aerated throughout the trials. No artificial lights were on during the trial nor were the buckets kept in direct sunlight. No attempt was made to darken the room further.

A stock solution of 2 g/L of commercially available chloramphenicol (Rochelle Laboratories, Long Beach, CA), furpyrinol (Abbott Pharmaceutical, North Chicago, IL), oxytetracycline (C. Pfizer & Co., Inc., Brooklyn, NY), and carrier-free erythromycin (Sigma Chemicals, St. Louis, MO), respectively, were prepared. Each was subsequently added to buckets containing 3.8 L of water so as to achieve dilutions in twofold progression from 4 mg/L to 32 mg/L. An additional untreated bucket served as a negative control. Fish remained in the water containing antibiotics for 5 h. At the end of the holding period three of the four fish were killed, weighed, and chopped; their tissues formed one pool. The fourth fish was pithed, incised ventrally, and a dacron swab inserted into the visceral cavity.

Chopped fish tissue was extracted for 45 min at weight: volume concentrations of 1:5, 1:10, and 1:20 using an appropriate solvent. The solvents used for each drug were chloramphenicol, 30% methanol; erthroymcin, 0.2 mol/L

phosphate buffer at pH 8; furpyrinol, 30% dimethyl sulfoxide; and oxytetracycline, 0.01 mol/L hydrochloric acid. After extraction the respective solvent was filtered using a prefilter of cellulose and a 0.45-μm membrane filter. The filtrates were collected and three aliquots of each filtrate were assayed using sterile techniques.

Qualitative assay for the presence of antibiotic in visceral fluids was done by inserting a dacron swab into the peritoneal cavity and leaving it in situ for 1 h. Swabs were removed and placed on *Bacillus subtilus* spore plates. The plates were incubated at 35°C for 18–24 h and examined. A zone of inhibition was recorded as an indication of absorption.

The antibiotic assay involved the inhibition of germination of spores from *B. subtilus* ATCC 6633. *Bacillus subtilus* was grown in bottles on trypticase agar for 4 d at 37°C, the cells harvested, spore concentration determined, and a stock solution prepared so as to achieve a spore concentration of $\sim 5.5 \times 10^5$ spores/mL of plating medium. Stainless steel assay cylinders having a capacity of 0.3 mL/per cylinder were placed on the agar surface. The extracted tissue filtrates were assayed using 0.25 mL/per cylinder. If necessary further dilutions of the filtrates were made and a subsequent assay done to estimate the antibiotic concentrations.

A separate aliquot of water from each bucket was drawn at the end of the trial. Drug decay (%) was determined from measurements of the initial and final concentrations in the water.

Reference curves were prepared using serial twofold dilutions of each antibiotic being studied. These dilutions were placed in stainless steel cylinders previously placed on *B. subtilus* spore plates. Each point on a curve was the mean of six to nine replicates of a given dilution. Values were plotted on five-cycle semilog paper with the concentration of antibiotic on the logarithmic scale and the diameter of the zone of inhibition recorded in millimetres on the arithmetic scale.

Results — Chloramphenicol was not detected in any of the fish by either the qualitative or extraction procedures. Residual concentrations were detectable in the water after 5 h and were ~15–20% of the amount originally put into the water (Table 1).

The tissue concentration of erythromycin progressed linearly with the concentration in the water. Tissue concentrations were approximately fourfold greater with each twofold increase in water concentration (Table 1). Degradation of erythromycin in water was slow, and from 60 to 87% of the original concentration remained after 5 h.

Roughly 75% of the water concentration of furanace was found in the tissues of the fish. At concentrations >4 mg/L, no antibiotic decay could be demonstrated during the 5-h test period (Table 1). Toxicity, manifested as easy excitability and erratic swimming, was seen in the 32 mg/L group.

Oxytetracycline residual in fish tissue varied from 46 to 52% of the drug available in the water. In assays of tissue from fish taken from the 4 mg/L water, 0.6 mg/g was recovered; only 32% of the original concentration of oxytetracycline remained after 5 h and this percentage was constant regardless of concentration (Table 1).

Table 1 shows that, based on the rate of absorption into channel catfish, the drugs could be ranked from most readily absorbed to least readily absorbed: furpyrinol, oxytetracycline, erythromycin, and chloramphenicol. (The latter was not demonstrated in tissue extracts.) A stability gradient in water of furpyrinol, erythromycin, oxytetracycline, and chloramphenicol would be established. The absorption efficiency of these drugs by channel catfish from water formed a gradient with furpyrinol first and erythromycin and oxytetracycline second. Qualitative swab studies verified the quantitative studies in each instance.

TABLE 1. Absorption of several antimicrobic drugs from water by channel catfish, *Ictalurus punctatus*, measured after 5 h using *Bacillus subtilus* spore germination inhibition assay

mg/L added to water	mg/kg in fish (%)[a]	mg/L in water	% drug decay in water
Chloramphenicol			
4	0(0)	0	100
8	0(0)	1.2	85
16	0(0)	3.5	78
32	0(0)	6.8	78
Erythromycin (carrier-free)			
4	0.6(15)	2.4	40
8	1.5(17)	7.0	13
16	4.0(25)	14.0	13
32	7.5(28)	27.0	13
Furpyrinol			
4	2.5(62)	3.6	10
8	6.0(75)	8.0	0
16	12.0(75)	16.0	0
32	17.5(56)	31.0	0
Oxytetracycline			
4	0.6(15)	1.3	68
8	1.3(16)	2.6	68
16	2.7(17)	5.2	68
32	5.2(17)	10.0	68

[a]Data are means of extrapolations from diluted aliquots and from replicate samples. In no instance did the range exceed ±2 mg/kg.

Discussion — The following broad statements can be made: chloramphenicol, despite its ease of absorption in mammalian systems over a wide pH range, does not appear to be of use in fish therapy other than via injection. We believe, however, that the drug might be used to reduce bacterial populations in water and perhaps lessen stress on fish therein. It was demonstrated by Aoki (1975) that chloramphenicol tended to reduce the development of resistance plasmid transfer in situations where oxytetracycline and a sulfamerizine have been used extensively (Aoki and Egusa 1971). The high MIC of chloramphenicol necessary for most common bacterial pathogens of fish would negate its use in feed even on a prophylactic basis (Nusbaum and Shotts 1981).

The marked utility of erythromycin for the control of bacterial kidney disease is based upon the specificity of the compound for Gram-positive organisms and that the drug is either injected or used in water hardening. When placed directly into water, erthromycin is absorbed by catfish at a rate equal to one-fifth to one-eighth of the MIC necessary to

inhibit most Gram-negative bacterial pathogens of fish.

Furpyrinol achieves MIC levels in catfish in a very short time at low concentrations (Dainippon Pharmaceutical Ltd., Osaka, Japan). Since the absorbed antibiotic concentration closely parallels the concentration added to the water, a dose-related response is achievable.

Oxytetracycline, although most effective in acid environments (pH 6–7) (Aronsom and Kirk 1974), is active over a broad pH range (6–9). From the data obtained in this study, oxytetracycline is absorbed at a rate of 15–17% of the water concentrations. This provides flexibility in dosage and allows this drug to be administered to correlate with the MIC of the particular bacterial organism causing disease in fish.

Unquestionably, the effect of water quality (temperature, pH, hardness, and flow rate), species of fish being treated, and the preparation of the drug used would alter the data presented here. This report should therefore serve as a format for those wishing to institute a quantified program of antimicrobial therapy. Not all of the potentially useful drugs have been tested nor can the efficacy of a given drug be assessed without a culture and sensitivity of the bacteria for which the drug is to be used. Also necessary is the physiochemical description of the impoundment.

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Absence of the Protozoan *Myxosoma cerebralis* (Myxozoa: Myxosporea), the Cause of Whirling Disease, in a Survey of Salmonids from British Columbia

L. MARGOLIS, T. E. MCDONALD, AND G. E. HOSKINS

Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

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Myxosoma cerebralis (Hofer, 1903) was not observed in 973 wild and 4496 cultured salmonids examined from collections made in widely separated localities in British Columbia from 1968 to 1980. Species investigated were *Salmo clarki* (180), *S. gairdneri* (3688), *S. trutta* (16), *Salvelinus fontinalis* (185), *S. malma* (300), *Oncorhynchus gorbuscha* (109), *O. keta* (17), *O. kisutch* (785), *O. nerka* (126), and *O. tshawytscha* (63). Previous reports notwithstanding, in the absence of corroborative evidence British Columbia (Canada) should not be accepted as a center of *M. cerebralis* infection.

Key words: *Myxosoma cerebralis*, salmonids, British Columbia, whirling disease

MARGOLIS, L., T. E. MCDONALD, AND G. E. HOSKINS. 1981. Absence of the protozoan *Myxosoma cerebralis* (Myxozoa: Myxosporea), the cause of whirling disease, in a survey of salmonids from British Columbia. Can. J. Fish. Aquat. Sci. 38: 996–998.

L'examen de 973 salmonidés sauvages et de 4496 salmonidés d'élevage dans des collections faites à des endroits très éloignés les uns des autres en Colombie-Britannique, de

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1968 à 1980, n'ont pas révélé la présence de *Myxosoma cerebralis* (Hofner, 1903). Les espèces étudiées étaient les suivantes : *Salmo clarki* (180), *S. gardneri* (3688), *S. trutta* (16), *Salvelinus fontinalis* (185), *S. malma* (300), *Oncorhynchus gorbuscha* (109), *O. keta* (17), *O. kisutch* (785), *O. nerka* (126) et *O. tshawytscha* (63). Nonobstant des rapports antérieurs, la Colombie-Britannique (Canada), ne devrait pas, en l'absence de preuves à l'appui, être acceptée comme centre d'infection par *M. cerebralis*.

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Myxosoma cerebralis (Hofer, 1903) Kudo, 1933 is widely distributed in Europe and the USSR and in recent years has been disseminated from central Europe to other parts of the world, including the USA (Hoffman 1970; Bogdanova 1973; Christensen 1973; Bauer 1975; Halliday 1976). The introduction and establishment of *M. cerebralis* in new geographic areas is a matter of considerable concern to fish culturists and fisheries resource managers because it is the cause of whirling disease, a serious ailment of cultured salmonids, particularly rainbow trout (*Salmo gairdneri*). In recent years, fish health protection regulations that include measures to prevent the introduction and spread of *M. cerebralis* have been established in a number of countries, including Canada.

Clinical whirling disease has not been observed in British Columbia (or elsewhere in Canada), but Bogdanova (1969, 1970a, b) claimed to have found low numbers of spores of *M. cerebralis* in wild Dolly Varden charr,[1] *Salvelinus malma*, from the Bella Coola River and its tributaries (central coast of British Columbia), and in pink salmon, *Oncorhynchus gorbuscha*, which she stated came from "southwestern Canada" (Bogdanova 1970b) or "western Canada" (Bogdanova 1977). Her pink salmon samples were actually collected in the southeastern Gulf of Alaska (Bogdanova 1970a, 1977), and the exact North American freshwater areas of origin of these fish are unknown. Based on the reports by Bogdanova, a number of review articles include Canada among the countries in which *M. cerebralis* occurs (Hoffman 1970; Lom and Hoffman 1970; Christensen 1973; Bogdanova 1973, 1974; Cordero del Campillo et al. 1975; Bauer 1975).

Following the appearance of Bogdanova's publications on the occurrence of *M. cerebralis* in British Columbia we undertook to confirm her results by examining wild Dolly Varden charr and other salmonids from the Bella Coola and other river systems in British Columbia. As noted by Halliday (1976), by 1972 we had examined over 500 wild salmonids, including 270 Dolly Varden charr from the Bella Coola River system, without detecting *M. cerebralis*. During the past 8 years we examined several thousand additional wild and cultured salmonids, belonging to 10 species of three genera, from widely separated localities in British Columbia (Table 1). Fingerlings to postspawned adults were included in the samples. Data on each sample examined and the method of its examination are provided in Margolis et al. (1981). From the Bella Coola River system a total of 646 fish of five species (95 *S. clarki*, 10 *S. gairdneri*, 296 *S. malma*, 145 *O. kisutch*, and 100 *O. gorbuscha*) has been examined.

TABLE. 1. Species and numbers of Salmonidae from British Columbia examined for *Myxosoma cerebralis*, 1968–1980.

Species	No. examined	
	Wild	Cultured
Salmo clarki	180	—
Salmo gairdneri	48	3640
Salmo trutta	16	—
Salvelinus fontinalis	30	155
Salvelinus malma	300	—
Oncorhynchus gorbuscha	109	—
Oncorhynchus keta	10	7
Oncorhynchus kisutch	175	610
Oncorhynchus nerka	105[a]	21
Oncorhynchus tshawytscha	—	63
Totals	973	4496

[a]Includes 50 smolts from the Klukshu River, Yukon Territory, north of the British Columbia boundary.

Samples were processed mainly by spore-concentrating techniques, although initially some were examined by more direct methods involving crushing skulls in a mortar (Hoffman et al. 1968) or between two large glass slides. In the earlier years the spore-concentrating technique described by Gillespie et al. (1974) was used, whereas in later years the digestion method of Markiw and Wolf (1974a, b) as detailed in Anon. (1977) was applied. Wild fish generally were examined individually and cultured fish were pooled in groups of 3 to 10. In all cases spores of *M. cerebralis* were not found.

A gross abstracting error in Protozoological Abstracts added to the confusion about the occurrence of *M. cerebralis* in Canada. The abstract (Anon. 1978) attributed to Christensen and Elleman (1976) a report of *M. cerebralis* in Pacific salmon from Canada. These authors refer to *M. cerebralis* as the cause of whirling disease in rainbow trout in Danish trout farms and make no comment about this organism in Pacific salmon. The error, clearly due to misunderstanding of the Danish text, was later corrected (Anon. 1979).

Earlier reports notwithstanding, the results of our extensive examinations of salmonids from British Columbia strongly indicate that *M. cerebralis* does not occur in this province. Since there are no records from other provinces, Canada should not be accepted as a focus of *M. cerebralis* infection without corroboration.

[1]The spelling differs from that (char) used in *A List of Common and Scientific Names of Fishes from the United States and Canada*, by the American Fisheries Society, and is preferred by the authors.

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Determination of Free Metal Ion Concentrations Using Bioassays

UWE BORGMANN

Department of Fisheries and Oceans, Great Lakes Biolimnology Laboratory, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

BORGMANN, U. 1981. Determination of free metal ion concentrations using bioassays. Can. J. Fish. Aquat. Sci. 38: 999–1002.

A procedure was developed by which free metal ion concentrations, toxic to aquatic life, can be calculated by comparing metal toxicity before and after addition of a complexing agent of known complexing capacity. Application of this method suggests that the growth of freshwater copepods is affected at free copper concentrations around 10^{-10} to 10^{-9} mol·L^{-1}. In natural waters, with unidentified ligands of unknown complexing ability, this procedure provides the only method currently available for estimating free metal concentrations at the low levels often causing sublethal toxicity to aquatic organisms.

Key words: metal toxicity, complexation, free metal, copepods, copper

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Le procédé décrit ci-dessous permet de calculer les concentrations d'ions métalliques libres toxiques pour la vie aquatique, en comparant la toxicité des métaux avant et après addition d'un agent complexant dont la capacité de complexation est connue. L'application de cette méthode donne à penser que des concentrations de cuivre libre d'environ 10^{-10} à 10^{-9} mol·L^{-1} influencent la croissance des copépodes. Dans des eaux naturelles, avec coordinats non identifiés et de capacité de complexation inconnue, c'est la seule méthode présentement disponible permettant d'estimer les concentrations de métaux libres aux faibles niveaux souvent responsables d'une toxicité sublétale chez les organismes aquatques.

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DIFFERENCES in the ability of natural seawaters to support aquatic life and the possible role of trace metals in producing these differences have been recognized for some time (e.g. Wilson and Armstrong 1961). The effect of metals is, however, related to their degree of complexation, and the importance of organic complexing agents is now well recognized (e.g. Barber and Ryther 1969; Steemann Nielsen and Wium-Andersen 1970; Barber 1973; Sunda and Guillard 1976; Anderson and Morel 1978; Sunda and Lewis 1978). It is generally believed that only noncomplexed free metal ions are toxic to aquatic organisms (Zitko et al. 1973; Pagenkopf et al. 1974; Sunda and Guillard 1976; Anderson and Morel 1978; Jackson and Morgan 1978; Sunda and Lewis 1978; Canterford and Canterford 1980). Free metal concentrations are, however, difficult to measure. Although metal ion electrodes are occasionally used (e.g. Zitko et al. 1973; Sunda and Lewis 1978), their sensitivity is limited. Free metal concentrations in toxicity studies are often calculated from equilibrium models and computer programs (e.g. Pagenkopf et al. 1974; Sunda and Guillard 1976; Andrew et al. 1977; Anderson and Morel 1978; Jackson and Morgan 1978; Van den Berg et al. 1979; Canterford and Canterford 1980). Because the concentrations of all complexing agents must be known for such calculations, this method is of limited use in studies of natural waters where unknown complexing agents may exist.

The purpose of this paper is to demonstrate a procedure for calculating free metal ion concentrations using bioassay data. The procedure is relatively simple, can determine free metal concentrations toxic at sublethal levels to aquatic organisms (i.e. below the detection limit of present physical chemical methods), and can be used when unknown complexing agents are present and modelling techniques are unsuitable. This method was used to calculate free copper concentrations affecting the growth of natural freshwater copepod populations.

Materials and methods — The procedure of Borgmann et al. (1979), as slighly modified by Borgmann (1980), was used to determine copepod production rates. Surface water from the Burlington Canal was filtered through 102- and 64-μm pore size nylon screens and the intermediate-sized nauplii were used to initiate experiments. Naupliar density was determined from a subsample and aliquots estimated to contain about 100 nauplii were placed in Pyrex glass jars with sufficient water, previously filtered through 44-μm mesh screens to eliminate all crustacean plankton, to make a volume of 2 L. Burlington Canal surface water usually originates from Hamilton Bay. As a comparison, experiments were also conducted in water from Lake Ontario, collected from the end of

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the breakwater projecting into the lake on the north side of the canal. After a 1-wk incubation at 20°C in a 12-h light:12-h dark photoperiod, the copepods were filtered through a series of varying sized nylon screens and counted. Mean size per individual was obtained from size frequency data. The instantaneous growth rate and copper concentration causing a 20% reduction in the growth rate were calculated by regression analysis as described in Borgmann et al. (1979, 1980).

Copper was added in the form of $CuCl_2 \cdot 2H_2O$ following the addition of nauplii and amino acids. Amino acids were obtained from Sigma Chemical Co. The medium was not stirred or changed during the incubation period. All calculations are based on copper concentrations added, not measured. Total copper concentrations were measured extensively in one experiment, and the mean measured concentration was observed to be 96% of added copper (coefficient of variation 24%). Measured copper concentrations were determined by atomic absorption spectrophotometry by the Water Quality Branch of the Canada Centre for Inland Waters.

Stability constants for copper amino acid complexes were obtained from Martell and Smith (1974). All stability constants were corrected to an ionic strength of 0.006 using the Davies equation (Stumm and Morgan 1970). An ionic strength estimate of 0.006 was obtained from measured calcium and magnesium concentrations of 56 and 12 $mg \cdot L^{-1}$, respectively, assuming all anions (mainly HCO_3^-) had a charge of -1. The values of the corrected stability constants used in the calculations are listed in Table 1.

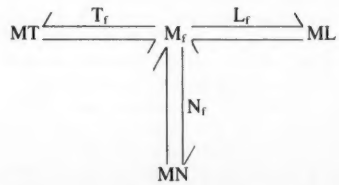
Theory — The free metal ion concentration can be calculated from the displacement of the toxicity curve after addition of a weak complexing agent with a known stability constant. The stability constant for a metal−ligand complex is given by

$$(1) \quad K = \frac{[ML]}{[M_f][L_f]}$$

where $[M_f]$ and $[L_f]$ are the concentration of the free metal and ligand, respectively, and [ML] is the concentration of the metal−ligand complex. Rearrangement gives

$$(2) \quad [M_f] = \frac{[ML]}{K[L_f]}.$$

Hence, $[M_f]$ can be calculated if [ML] and $[L_f]$ are known. [ML] can be obtained by consideration of the following equilibria



where T represents ligands which form toxic complexes with the metal, N represents ligands which form nontoxic complexes with the metal, and L represents a known added nontoxic ligand. Because the nature and concentrations of T_f and N_f in natural waters are not usually known, the contribution of MT to overall toxicity may be difficult to determine. The total concentration of metal is given by

$$(3) \quad [M_T] = ([M_f] + [MN] + [MT]) + [ML].$$

For any one water sample, the total concentrations of N and T are constant and therefore [MN] and [MT] depend only on $[M_f]$. If $[M_f]$ remains constant, [MN] and [MT] must remain constant and hence toxicity must also remain constant (assuming ML is nontoxic). It follows that [ML] and total metal concentrations must increase by equivalent amounts if $[M_f]$, [MT], and [MN] (and toxicity) remain constant. Therefore, [ML] can be determined for any growth rate by subtracting the total metal concentration giving that rate without added ligand from the total metal concentration giving the same growth rate in the presence of the ligand. It should be noted that equation (2) permits only the calculation of free metal concentrations and not toxic metal concentrations (i.e. $[M_f] + [MT]$), even though the toxicity curve is the result of both M_f and MT.

Equation (2) can be used for determining $[M_f]$ whenever the complex ML predominates, but not if complexes such as ML_2 predominate, as is sometimes the case with amino acids. The equivalent of equation (1) for such a complex is given by an alternative stability constant

$$(4) \quad \beta_2 = \frac{[ML_2]}{M_f[L_f]^2}$$

and therefore equation (2) should be replaced with

$$(5) \quad [M_f] = \frac{[ML_2]}{\beta_2[L_f]^2}.$$

The value of $[L_f]$ in equations (2) and (5) can be determined from

$$(6) \quad [L_f] = a([L_T] - \Sigma\, n[ML_n])$$

where a is a correction factor to take into account the degree of complexation of the ligand by other metals in the water. The value of a may be calculated from

$$(7) \quad a = \frac{[L_f]}{[L_f] + \Sigma\,[X_iL]}$$

where X_i represents calcium, magnesium, hydrogen ion, and any other cations which significantly bind to L.

An important consequence of equations (2) and (5) is that free metal concentrations can be calculated only from experimental data employing weak complexing agents (i.e. metal ion buffers). In this case, L_T is in much greater concentrations than ML_n, and $[L_f]$ can readily be calculated. For strong complexing agents $[L_T] - \Sigma\, n[ML_n]$ is much smaller than $[L_T]$, making the use of equation (6) inaccurate. Hence, free metal ion concentrations cannot usually be calculated using the more popular chelating agents such as EDTA.

TABLE. 1. Values for the stability constants (from Martell and Smith 1974) after correction to an ionic strength of 0.006 as used in the calculations of free copper concentrations. The form of the non-ionized amino acid is HL for glycine and H_2L for glumatic acid. Charges are omitted for simplicity.

Stability constant	log K or β	
	Glycine	Glutamic acid
$K_1 = \frac{[CuL]}{[Cu][L_f]}$	8.42	8.47
$\beta_2 = \frac{[CuL_2]}{[Cu][L_f]^2}$	15.43	14.76
$\frac{[HL]}{[H][L_f]}$	9.71	9.81
$\frac{[MgL]}{[Mg][L_f]}$	2.52	2.50
$\frac{[CaL]}{[Ca][L_f]}$	1.25	1.78
$\frac{[CaHL]}{[Ca][HL]}$	—	1.03

Results and discussion — The observed changes in the copper concentrations required to cause a 20% drop in growth rate of freshwater copepods (ΔG20) and the free copper ion concentrations calculated from these changes are summarized in Table 2. For two dates, toxicity curves in the presence and absence of amino acids were not obtained at the same time, as they theoretically should have been, and these values are enclosed in parentheses. To obtain the estimates of free copper ion concentrations, the value a (equation (7)) was first determined. From the hydrogen ion (pH = 8.1 ± 0.1) and calcium and magnesium concentrations the ratios of [HL], [CaL], [MgL], and for glutamate [CaHL], to $[L_f]$ are readily obtained using the equilibrium constants in Table 1. This gives $a = 0.0218$ for glycine and $a = 0.0172$ for glutamate, with the bulk of the amino acid being in the HL form. Because the estimated amount of copper–amino acid complex (ΔG20, Table 2), is much less than the total added ligand concentration (100 μmol·L^{-1}), this provides an initial estimate of free ligand concentrations of 2.18×10^{-6} mol·L^{-1} for glycine and 1.72×10^{-6} mol·L^{-1} for glutamate. Dividing β_2 (Table 1) by K_1 and multiplying by L_f gives the ratio $[CuL_2]/[CuL]$, which equals 22.3 for glycine and 3.35 for glutamate. Hence the concentration of the dominant complex, CuL_2, can be determined assuming $[CuL_2] + [CuL] = \Delta G20$. Because $[CuL_2]$, and therefore also [CuL], are known, the estimate of free ligand concentration can be further refined using equation (6), and this in turn permits calculation of free copper concentrations using equation (5).

In the above calculations, it is assumed that the copper–amino acid complexes are nontoxic. This was verified in experiments which showed that increasing the concentration of glycine or glutamic acid from 0 to 320 μmol·L^{-1} gradually reduced the toxicity of 100 or 200 μg·L^{-1} of added copper to values approaching the control (with amino acid but without copper, unpublished data). The amino acids themselves had little effect on copepod growth rates. It cannot, however, be assumed that all amino acid complexes are nontoxic. Histidine, for example, reduced copper toxicity to copepods only partially, suggesting the copper–histidine complex was toxic (unpublished data).

The experiments reported here were initially designed to investigate the effect of complexing agents on copepod production, rather than to calculate free metal concentrations per se. This has resulted in one drawback of the present study, namely that the lengthy (1 wk) incubation periods required may have resulted in altered amino acid concentrations due to microbial activity. Although this may have some impact on the calculated free metal concentrations, the values in Table 2 compare favorably with free copper concentrations toxic to phytoplankton as calculated using computer modeling techniques and ranging from 3×10^{-11} to 10^{-8} mol·L^{-1} (Sunda and Guillard 1976; Anderson and Morel 1978; Jackson and Morgan 1978; Van den Berg et al. 1979; Canterford and Canterford 1980). Anderson et al. (1977), also using a modeling technique obtained 50% survival of *Daphnia magna* in 24 h at ~10^{-8} mol·L^{-1} free copper. Because this was a lethal test, toxic free copper concentrations would be expected to be higher than for the sublethal test reported here (Table 2).

The most significant feature of the free metal determinations summarized in Table 2 is that free copper concentrations could not have been estimated by any other means. Metal ion electrodes are not yet sufficiently sensitive for routine use at the concentration ranges indicated in Table 2, and techniques such as differential pulse anodic stripping voltammetry measure only labile metal, which includes both free metal and metal bound to weak complexing agents (Chau et al. 1974). Free metal concentration estimates in toxicological studies have therefore often been obtained by mathematical modeling techniques, using computer programs and published stability constants for the various complexes formed. This method is dependent on the accuracy of a large

TABLE. 2. Copper concentrations causing a 20% reduction in growth rate (G20) with (+L) and without (−L) 100 μmol·L^{-1} glycine or glutamic acid, concentration of the CuL_2 complex, and log of the calculated free copper ion concentration.

Date	Water source	Ligand	G20(−L) μg·L^{-1}	G20(+L) μg·L^{-1}	ΔG20 μmol·L^{-1}	$[CuL_2]$ μmol·L^{-1}	log $[Cu^{2+}]$ μmol·L^{-1}
June 10/80	Hamilton Bay	Glycine	29.7	61.0	0.49	0.47	−10.43
Aug. 1–Aug. 28	Hamilton Bay	Glutamate	33.2	76.5	(0.68)	(0.52)	(−9.50)
Sept. 11	Hamilton Bay	Glutamate	76.6	140.3	1.00	0.77	−9.33
July 31–Aug. 5	Lake Ontario	Glycine	19.7	136.3	(1.88)	(1.78)	(−9.82)
Sept. 24	Lake Ontario	Glutamate	8.2	68.0	0.94	0.72	−9.36

number of published stability constants,and can only be employed if the concentrations of all complexing agents are known, thereby usually limiting its use to defined artificial media and making this method unsuitable here. Modeling techniques should be used cautiously for natural waters where unknown ligands may occur. McCrady and Chapman (1979), for example, observed that free copper concentrations in several natural waters measured by copper ion electrode were nearly always lower than those estimated from mathematical models, especially at low copper concentrations.

The technique for measuring free metal concentrations described here (equations (2), (5), and (6)) has several advantages. It is mathematically much simpler than complete modeling techniques and requires accurate knowledge of only a few stability constants. It can be used in either natural waters or artificial media and has no limit to sensitivity as long as an organism responding to metal concentrations in the range of interest is available. Although growth was used in the present study, any quantifiable response of an organism to metals can be used as it is only total metal concentrations that give equivalent responses that need be determined. The decrease in growth rates due to limitation of essential metals could therefore be used to quantify free metal concentrations at very low levels.

The above method is somewhat analogous to the method of determining free metal using metal ion electrodes calibrated against metal in a metal buffering solution (e.g. acetate buffer for Cu^{2+}) (McCrady and Chapman 1979). The only differences are the use of an organism to obtain the measured response and the addition of the metal buffer (i.e. weak complexing agent) to the water in question, rather than to a pure standardization medium. This latter change is required because no food source is available in pure medium and because the organism, unlike the electrode, may respond to complexes of some ligands, which therefore must be held constant. In this way, only the free metal ion concentration is being determined, without interference from unknown toxic complexes. Huisman and Hoopen (1978) also added a weak complexing agent (*N*-methyliminodiacetic acid), at concentrations exceeding total metal concentrations, to obtain known concentrations of free metal, in this case to study the effect of Hg^{2+} on algae. However, they calculated free metal from total metal concentrations rather than the concentration of the metal ligand complex. They assumed total metal equaled free metal plus metal complexed to the added ligand and ignored possible complexation of metal with other ligands, making their procedure less suitable for use in natural waters.

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On Estimating Partial Recruitment in Virtual Population Analysis

DEREK A. ROFF

Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Que. H3A 1B1

ROFF, D. A. 1981. On estimating partial recruitment in virtual population analysis. Can. J. Fish. Aquat. Sci. 38: 1003–1005.

Virtual population analysis or cohort analysis is hindered by the difficulties of estimating the partial recruitment factors. A method currently used for estimating the latter is the ratio of the frequency of a particular age in the commercial catch to that in a research sample. This method is examined and shown to be only an approximation that may cause significant errors in the analysis. An alternate method, utilizing the same data, is proposed. A method is then given that tests the assumption that the research sample is a random sample from the population.

Key words: population estimation, cohort analysis, virtual population analysis, partial recruitment

ROFF, D. A. 1981. On estimating partial recruitment in virtual population analysis. Can. J. Fish. Aquat. Sci. 38: 1003–1005.

Les difficultés que pose l'estimation des facteurs de recrutement partiel nuisent à l'analyse de populations virtuelles ou de cohortes. On utilise présentement le rapport entre la fréquence d'un âge particulier dans les prises commerciales et celle d'un échantillon par navire de recherche dans l'estimation de ces facteurs. Nous examinons cette méthode et démontrons qu'elle n'est qu'une approximation qui peut être cause d'erreurs importantes dans l'analyse. Nous proposons une autre méthode tout en utilisant les mêmes données. On indique ensuite comment on peut vérifier l'hypothèse que l'échantillon d'un navire de recherche est un échantillon aléatoire de la population.

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A much used tool for stock assessment is virtual population analysis or its simplified version, cohort analysis. One of the major stumbling blocks in its use is the estimation of the terminal fishing mortality required to initiate the calculations. A secondary though equally troublesome problem is the estimation of partial recruitment factors for those stocks in which recruitment to the "catchable" population is not an all-or-none phenomenon, i.e. some age- or size-groups are less vulnerable to the fishing gear than others. One estimate of the partial recruitment factor that has been proposed is the ratio between the frequency of a particular age-group in the commercial catch to the frequency of the same age-group in the research catch (Winters 1978a, b). This method has been used for harp seals, *Pagophilus groenlandicus* (Winters 1978a), and for a variety of marine fish species, *Hippoglossoides platessoides*, *Gadus morhua*, *Sebastes* sp., *Clupea harengus*, and *Scomber scombrus* (unpublished data from assessments presented at the 1980 CAFSAC meeting). In this paper I shall examine the validity of this method and propose an alternate method that is preferable because the former is biased. Both methods assume that the research catch represents a random sample from the population. A method for testing this assumption will be presented.

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The ratio method — By the ratio method we have

$$(1) \quad \hat{R}_t(a) = \frac{f_t'(a)}{f_t(a)\,\hat{R}_t^*}$$

where $\hat{R}_t(a)$ = estimated partial recruitment multiplier for age a at time t; $f_t(a)$ = frequency of age-group a in the research catch at time t (it is assumed that the research catch is a random sample from the population); $f_t'(a)$ = frequency of age-group a in the commercial catch at time t; $\hat{R}_t^*$ = maximum observed value of $\hat{R}_t$. In this section I show that this estimate is a biased estimate of $R_t(a)$.

From the catch equation we obtain the numbers of fish of age a caught at time t, $C_t(a)$,

$$(2) \quad C_t(a) = \frac{N_0 f_t(a)\, F_t R_t(a)\,(1 - e^{-(F_t R_t(a) + M)})}{F_t R_t(a) + M}$$

where N_t = population size at time t; $f_t(a)$ = frequency of age-group a in the population and research catch at time t; F_t = fishing mortality on fully recruited cohorts; $R_t(a)$ = partial recruitment multiplier for age a at time t; M = natural mortality rate. Now

$$(3) \quad f_t'(a) = C_t(a)/C_t$$

where C_t = the total catch at time t. Under the assumption of the ratio method we have

$$(4) \quad R_t(a) \propto \frac{f_t'(a)}{f_t(a)} = \frac{f_t(a)\, N_t F_t R_t(a)\, (1 - e^{-(F_t R_t(a) + M)})}{C_t f_t(a)\, (F_t R_t(a) + M)}$$

Because the maximum value $R_t(a)$ can take is 1, we divide throughout by the maximum value obtained from equation (4), R_t^*,

$$(5) \quad R_t^* = \frac{N_t F_t\, (1 - e^{-(F_t + M)})}{C_t\, (F_t + M)}.$$

Hence the relationship between $\hat{R}_t(a)$ and $R_t(a)$ is

$$(6) \quad \hat{R}_t(a) = \frac{R_t(a)\, (1 - e^{-(F_t R_t(a) + M)})\, (F_t + M)}{(F_t R_t(a) + M)\, (1 - e^{-(F_t + M)})}.$$

The ratio method provides a biased estimate of $R_t(a)$ because algebraically the above equation does not balance ($\hat{R}_t(a) \neq R_t(a)$). How bad might this bias be? For small values of x, $1 - e^{-x}$ is approximately equal to x and hence as $(F_t R_t(a) + M) \to 0$, and $(F_t + M) \to 0$, $\hat{R}_t(a) \to R_t(a)$. Thus, if fishing mortality and natural mortality are very low, equation (6) will be relatively unbiased.

Also, as $R_t(a) \to 1$, $F_t R_t(a) + M \to F_t + M$ and $\hat{R}_t(a) \to R_t(a)$ and hence the bias will also be relatively small for cohorts almost fully recruited. This leaves us with intermediate to high mortality rates to consider and the instances of relatively incomplete recruitment. Some idea of the potential bias can be gained for these cases from the following example. If $M = 0.3$ and $F_t = 0.7$, then the values of $\hat{R}_t(a)$ when $R_t(a)$ equals 0.5 and 0.1 are 0.582 and 0.132, respectively. In the first instance the proportional error is ~15% and in the second 30%. The most important consideration is how these incorrect estimates translate into population estimates. The estimated population size is given by the formula

$$(7) \quad N_t(a) = \frac{C_t(a)\, (F_t R_t(a) q + M)}{F_t R_t(a) q\, (1 - e^{-(F_t R_t(a) q + M)})}$$

where $\hat{R}_t(a) = R_t(a) q$.

Consider now the ratio, predicted cohort size over cohort size, Q,

$$(8) \quad Q = \frac{(F_t R_t(a) q + M)\, (1 - e^{-(F_t R_t(a) + M)})}{q(F_t R_t(a) + M)\, (1 - e^{-(F_t R_t(a) q + M)})}.$$

The approximate value of Q will be $1/q$. In the example given above $Q = 0.88$ and $1/q = 0.86$ when $R_t(a) = 0.5$, and $Q = 0.76$ and $1/q = 0.76$ when $R_t(a) = 0.1$. In the first instance the predicted population size is underestimated by ~12% and in the second by 24%. Thus, there are instances where the ratio method may significantly underestimate the true population size. However, there are many cases in which the error is small and as a "quick and dirty" method of estimating the partial recruitment multiplier the ratio method might be quite satisfactory. But partial recruitment multipliers are required for cohort analysis and the computations for this are almost always done on a digital computer; therefore there is no need to settle for a quick inexact solution as the power of the computer can be brought to bear on the matter and an accurate estimate made through solution of equation (6) by numerical methods. This approach would not be necessary if a method of estimating partial recruitment exactly was available. In the next section I present such a method.

TABLE 1. Test for nonrandom sampling of male moulting harp seals. ($G = 21.3$, $P > 0.05$.)

Age in 1969	No.	Frequency %	Age in 1973	No.	Frequency %
3	25	12.5	7	23	17.6
4	19	9.5	8	29	22.1
5	30	15.0	9	22	16.8
6	26	13.0	10	16	12.2
7	28	14.0	11	19	14.5
8	38	19.0	12	13	9.9
9	34	17.0	13	9	6.9
	200			131	

The partitioning method — Members of different cohorts, by virtue of their different sizes, different location, or whatever, have different probabilities of being captured. However, this difference in risk may be eliminated by partitioning the cohorts into two portions, a portion that has entered the catchable population and a portion that is still unrecruited. Consider the following simple but extreme example: let the probability of being captured in a trawl be 1 and 0.5 for two cohorts. If the second cohort is partitioned into two equal groups then those in one group may be considered to have the same probability of capture as all the members in the first cohort. Let the proportion of the cohort fully at risk be $p(a)$. The problem is to estimate $p(a)$ and hence the appropriate partitioning factor for each cohort. This can be achieved given data from the commercial catch and from a research survey as with the ratio method. The expected number of cohort "a" in the commercial catch at time t is

$$(9) \quad C_t(a) = N_t f_t(a) p_t(a) \frac{F_t\, (1 - e^{-(F_t + M)})}{F_t + M}$$

where F_t = the fishing mortality of the fully recruited segment, which now has the same value for all cohorts because of the partitioning of the population into catchable and noncatchable segments. Rearranging equation (9) we have

$$(10) \quad p_t(a) = \frac{C_t(a)}{f_t(a)} \frac{(F_t + M)}{(1 - e^{-(F_t + M)}) F_t N_t}$$

or

$$(11) \quad p_t(a) \propto \frac{C_t(a)}{f_t(a)} \text{ or } p_t(a) \propto \frac{f_t'(a)}{f_t(a)}.$$

Note the similarity between the above relationship and equation (4). However, whereas in equation (4) the proportionality between right- and left-hand sides was presumed, in the present case it is derived. Because the maximum value $p_t(a)$ can take is 1, we divide throughout by the maximum value

obtained from equation (10), p_t^*, and the estimate of $p_t(a)$, $\hat{p}_t(a)$, is

$$(12) \quad \hat{p}_t(a) = \frac{f_t'(a)}{f_t(a)\hat{p}_t^*}.$$

In estimating $p_t(a)$ from data the usual problems associated with the estimation of ratios should be taken into consideration (for a full discussion of these see Cochran (1977)).

The partial recruitment multiplier $R_t(a)$ is related to the partitioning factor $p_t(a)$ in the following manner:

$$(13) \quad e^{-(F_t R_t(a)+M)} = p_t(a)e^{-(F_t+M)} + e^{-M}(1 - p_t(a))$$

and hence

$$(14) \quad R_t(a) = \frac{-1}{F_t} \log_e (p_t(a)e^{-F}t + 1 - p_t(a)).$$

The initial population size can be calculated from

$$(15) \quad N_t(a) = \frac{C_t(a)\,(F_t + M)}{F_t(1 - e^{-(F_t+M)})\,p_t(a)}$$

or by calculating $R_t(a)$ and using the more usual formula.

A critical assumption in both methods is that the research catch takes a random sample of the population. If several years of research samples are available this assumption can be tested.

Testing the assumption of random sampling — Consider two cohorts "a" and "$a + 1$"; let their relative frequencies be $f_t(a)$ and $f_t(a + 1)$, respectively ($f_t(a) + f_t(a + 1) = 1$). If both cohorts are fully recruited then

$$f_t(a) = f_{t+j}(a), \qquad f_t(a + 1) = f_{t+j}(a + 1)$$

where $t + j$ is j yr after year t. If cohort a is not fully recruited and $a + 1$ is fully recruited, then the above equalities will not hold because the relative mortality rates will be different. If the research sample is a nonrandom sample of the population, the above equalities will also not hold because the relative susceptibilities to capture will likely be different in different years. Thus, if we restrict our attention in the research catch to those cohorts for which there is good evidence of full recruitment, we can examine the assumption that the research catch is a random sample from the population by comparing the relative frequency of cohorts as they age. The simplest way to do this is to compare frequency distributions across years with the χ^2 or G statistic: significant heterogeneity indicates nonrandom sampling. An example is presented in Table 1. These data are shot samples of male moulting harp seals; such samples have in the past been presumed to represent random samples from the population (Winters 1978a). Age-groups 1 and 2 were excluded as observation suggests that these may be nonrandomly sampled (D. Bowen, Fisheries and Oceans, St. John's, Newfoundland, personal communication). There is considerable heterogeneity between samples, age-groups 3 and 4 (1969 sample) being relatively overrepresented in the 1973 sample and age-groups 8 and 9 (1969 sample) being relatively underrepresented in 1973. To use either one of these to construct a set of partioning factors might therefore give highly erroneous results.

A research sample may be nonrandom in two ways. First, there may be systematic bias as might occur if susceptibility to capture were age specific, and second, there may be unsystematic bias due to chance or the method of collection. In either instance some function might be assumed for the relationship between the partitioning factor and age, and the data used to estimate the various parameters.

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BOOK REVIEWS/COMPTES RENDUS

Book review editor:
David P. Scott
Department of Fisheries and Oceans
Freshwater Institute
501 University Crescent
Winnipeg, Man. R3T 2N6

Aquatic Oligochaete Biology. *Edited by Ralph O. Brinkhurst and David G. Cook. Plenum Press, New York, NY. 1980. ix + 529 p. $55.00 U.S.*

This volume is a compilation of papers presented at the First International Symposium on Aquatic Oligochaete Biology held in Sidney, British Columbia, Canada, on May 1–4, 1979. The symposium brought together many of the most active workers in the field from throughout the world. This volume of proceedings represents the first compendium of international scope devoted to aquatic oligochaete biology. To students of the Oligochaeta, the impressive list of contributors and the variety of topics addressed should be sufficient justification for inclusion of this volume in many personal libraries. The volume comprises 26 papers by 34 contributors and presents both review-type information and new material that is arranged within five sections: Taxonomic Studies, Zoogeographic Studies, Life History and Production, Ecological Studies, and Oligochaetes in Relation to Human Activity. Several papers within each section present excellent state-of-the-art information that will greatly influence the approach and direction of future studies in the field.

Noteworthy in the section on Taxonomic Studies is the work of Grassle, who discovered sibling species of *Capitella capitata*, and Loden and Harman, who demonstrated the existence of ecologically induced phenotypic variation among the Naididae. Results of these two studies will likely have a profound effect on future attempts to define taxonomic limits of species and may result in a more conservative approach to elevation of subspecies, ecospecies, or variant forms to the species level.

Zoogeographic Studies presents the in-depth findings of investigators from the USSR, Argentina, USA (St. Lawrence Great Lakes), and England. The section highlight is a review by Timm of the worldwide distributional ranges of freshwater and marine families; included is a discussion of the evolution of aquatic oligochaetes and their distributional mechanisms and barriers. Spencer's account of the oligochaetes of the St. Lawrence Great Lakes is an excellent review of one of the most thoroughly studied regions in North America.

The Life History and Production section includes four papers. Poddubnaya presents findings on the life history of three widely distributed species in the USSR, *Isochaetides newaensis*, *Tubifex tubifex*, and *Limnodrilus hoffmeisteri*. Bonomi and Di Cola present a new model for estimation of production and population dynamics of *T. tubifex*. The life histories in both papers are presented in great detail, summarizing results of studies conducted during the last 10 years. Findings of Poddubnaya, and Bonomi and Di Cola demonstrate a longer, much more complex reproductive life history than was previously assumed. The section on Life History and Production ends with a paper by Stander on production biology of terrestrial Enchytraeidae. This paper not only provides the aquatic ecologist with an appreciation of the special problems confronting terrestrial oligochaetologists, but also presents results that may influence use of production to biomass ratios in aquatic studies.

Ecological Studies (seven papers) centers on factors influencing the distribution and abundance of oligochaetes in freshwater, estuarine, and marine environments. Interest in estuarine and marine oligochaetes has greatly increased in recent years. Excellent papers by Diaz, Birtwell and Arthur, and by Giere include much new and review material that addresses the growing wealth of information on estuarine and marine oligochaetes. Also, in this section is a review of extensive research on the influence of tubificids on geophysical and geochemical properties of Lake Erie sediments by McCall and Fisher

The final section within this volume (Oligochaetes in Relation to Human Activity) presents global approaches to the use of oligochaetes in various aspects of pollution biology. Milbrink offers an interesting review of attempts at lake typology based upon structure of oligochaete assemblages, and reviews tolerance and preference information on several widely distributed species. Milbrink also demonstrates the utility of a Modified Environmental Index (based upon Howmiller and Scott's 1977 Environmental Index) in the evaluation of trophic status. Lang and Lang-Dobler developed three indices of trophy based upon distributional patterns of oligochaetes in Lake Geneva, Switzerland. These biological indices of trophic status were then correlated to sediment chemical parameters. The approaches presented within these two papers may create a renewed interest in lake typology. Such interest may lead to continued development and refinement of trophic indices that incorporate autecological information on tolerance and preference rather than merely on community structure, as has been done with the several indices of diversity. Another noteworthy paper within this section is the work of Chapman et al. that deals with oligochaetes and their relationship to heavy metals. The relationship of heavy metals and oligochaetes has been undefined previously, and for years it was assumed that oligochaetes were relatively intolerant to heavy metals. This pioneering work of Chapman et al. demonstrates a large degree of tolerance and documents bioaccumulation of metals by worms.

Aquatic Oligochaete Biology will be a valuable reference for many scientists with interest and involvement in aquatic oligochaete taxonomy and ecology. While few investigators possess a scope of interest or expertise as varied as the topics addressed within this volume, certainly the contents of selected sections will justify adding this book to one's personal collection. North American investigators will likely be particularly appreciative of this work because of the abundance of European research that is presented and summarized. Organizers of the First International Symposium are to be commended for organizing a forum of this magnitude, and the editors deserve credit for overcoming what were likely many

difficulties in translating from the many languages.

HOWMILLER, R. P. AND M. A. SCOTT. 1977. An environmental index based on relative abundance of oligochaete species. J. Water Pollut. Control Fed. 49: 809–815.

K. STIMPSON

Ecological Analysts,Inc.
1500 Frontage Road
Northbrook, IL 60062, USA (R-328)

Epithelial Transport in the Lower Invertebrates. *Edited by B. Lahlou. Cambridge University Press, New York. 1980. xiv + 366 p. $55.00 U.S.*

This volume presents a synthesis of communications from a memorial symposium honoring the well-known comparative physiologist, Jean Maetz. The subject matter is presented at an advanced level and deals specifically with ionic transport in fishes and, to some extent, amphibians. Conveniently, the book is divided into four major sections: (1) morphological aspects; (2) transport of water and solutes; (3) biochemical aspects; (4) endocrine regulation. These sections are subdivided further into very specific units, e.g. "The ionocyte chalone: a chemical regulator of chloride cell proliferation and transformation" by F. P. Conte. The present state of knowledge in each of these areas is covered by leading investigators in the field. Much experimental evidence is presented in the communications which will be of great interest to those presently involved in this field. Casual readers, however, may be overwhelmed by the vast amount of specific data included. References, included at the end of each article, are extensive and will prove very helpful to readers wishing to investigate an area further. Of 29 papers presented in this book, 5 are written in French. Much of the information contained within each article, first presented in 1978, has since been published in scientific journals. Furthermore, a similar symposium honoring Jean Maetz was held in St. Louis at the 1978 annual meeting of the American Physiological Society. Proceedings from this symposium also were published as a special issue of the *American Journal of Physiology* and priced much more reasonably than this book. I suspect, however, that many persons would consider both publications as essential items for one's library, even considering the overlap between them.

The section describing transport of water and solutes is perhaps the most interesting and will prove very useful to those readers interested in modifiers of epithelial permeability and ionic exchange. A paper by W. T. W. Potts titled "The effects of pH and calcium on gill potentials and sodium balance in the brown trout, *Salmo trutta*" is not only interesting from a theoretical viewpoint but is also of practical importance considering recent effects of acid rain on many lakes. Potts suggests that fish gills are extremely permeable to H^+ ions and in fact may behave like hydrogen ion electrodes. This would explain the reversal of the trans-epithelial potential (TEP) from -16 to $+18$ mV in very acid media. Cause of death in acidic water is often attributed to sodium depletion. The loss of body sodium now can be explained by an increase in branchial Na^+ efflux and a reduction of Na^+ influx caused by a shift of TEP in the positive direction.

In recent years the opercular epithelium of *Fundulus heteroclitus*, a membrane rich in chloride cells, has been utilized for biophysical studies of salt transport in saltwater fish. Two interesting accounts of this preparation are provided: "Ionic exchanges in the opercular membrane of *Fundulus heteroclitus* adapted to seawater" by N. Mayer Gostan and Jean Maetz and "Chloride active transport and osmoregulation" by J. A. Zadunaisky and K. J. Degnan. Both papers present compelling evidence that chloride cells perform a purely active Cl^- secretory function while transport of Na^+ is almost exclusively passive and extracellular (paracellular). Cl^- secretion, in fact, may generate a driving force for a net Na^+ extrusion. Equally interesting are the descriptions of the actions of catecholamines on the opercular epithelium. Cl^- secretion apparently is controlled by stimulatory β receptors and inhibitory α receptors, β agonists increasing Cl^- secretion and α agonists decreasing Cl^- secretion. These effects prove that catecholamines probably act directly on the gill chloride cells and not secondarily by altering the branchial hemodynamics.

This book is not a general review of epithelial transport in the lower vertebrates and as such the title is somewhat misleading. It deals primarily with new trends in specific areas of research and, therefore, a fair amount of background knowledge is required to understand the material covered. This book is best suited for researchers investigating similar areas, but also may be valuable to advance students wishing more detail than general texts canprovide.

DAVID RANDALL

University of British Columbia
Department of Zoology
Vancouver, B.C.
V6T 1W5 (R-345)

Predator–Prey Systems in Fisheries Management. *Edited by Henry Clepper. Sport Fishing Institute, Washington, D.C. 1979. viii + 504 p. $20.00 U.S.*

Before reading this book, I used to wonder why any scientist concerned with ecological relationships would choose to study fish population processes, population dynamics, and communities. Fish are not easily observable by that terrestrial creature, man. The geographical scale of their interactions is often of such an order that it exceeds available methods. The consequences are not only unknown but seem unknowable. But now I know why. It is like mountain climbing. One studies fish because they are there and they are clouded in mystery. That mystery provides a marvelous opportunity for release of imagination for theory building unconstrained by reality. Fish ecology has generated such examples of sterile theory divorced from reality.

This symposium volume is refreshingly free of that. The contributed chapters are largely guided by ecologically significant questions and are rooted in empirical analysis and experiment. Theory is there certainly, as is modeling, but it is there as servant to the questions.

The purpose of the symposium was to contribute to an overall understanding of predator–prey systems, within fish populations, in a way that is useful for fisheries management.

That goal requires an understanding of how predation functions in a causative sense; of how predation interrelating with other processes such as competition affects population and community structure and behavior, and how fisheries operate and are or could be managed.

Functional analysis of the process of predation is well represented. Durbin provides a clear analysis of the action and interaction of components determining the causative structure of plankton-feeding fish and relates that to an evolutionary trend leading to a continuum of different feeding strategies. Optimal foraging theory is enthusiastically espoused, most notably by Ringler in considering selection of benthic prey, and by Werner in an effective analysis of partitioning of food size in fish communities. These examples show the two modes of functional or process analysis. The one emphasizes a description of what foraging is by identifying components and their interrelations. The other emphasizes what foraging should be, given an objective function of maximizing net energy gain. Both approaches are strongly empirical and share, moreover, a common starting point of organization by focusing on the sequence of acts in predation — search, pursuit, handling time, and so on. In the descriptive functional analyses that starting point is used to connect to behavioral and physiological knowledge at a lower level in the hierarchy of causation. In the "optimality" driven functional analyses, it is used to interpret higher levels in that hierarchy (questions of community structure and resource partitioning).

Both Larkin, in his beginning overview, and Rothschild and Forney, in their concluding summary, point to such functional analyses as fruitful avenues for future research. They both note, however, the expense of obtaining data, with Larkin remarking that possible costs might exceed the benefits, and Rothschild and Forney remarking that the benefits could well compensate the costs! As the relevant papers indicate, the unraveling of causation in each specific instance can be expensive and time-consuming. But the step needed to transfer such analyses to useful principles for understanding and for management is one of functional synthesis, not just analysis. It is clear from these papers, and others in the literature, that generality lies behind the specifics and that a finite set of classes of predation responses, distinguished by their form, can be classified together with the conditions for their existence. Given knowledge of the form, the demands for data are remarkably relaxed. Moreover, the optimal foraging literature suggests ways to classify strategic classes of predation distinguished by a defined range to parameter values. Those steps of synthesis lead to simplification which is the hallmark of understanding and of management usefulness.

But understanding of how predation functions is only a step to determining how predation affects population behavior and community structure. Despite the wealth of past work on fish population dynamics, there is surprisingly little exploration of population behavior in this book. There are useful examples of how predator/prey ratios affect size distributions in the papers by Hackney and Jenkins that illuminate the seminal notions of balance originated by Swingle. But there is no connection between those demonstrated effects and the understanding that has emerged from functional analyses. An example of filling that gap between causative understanding and behavioral consequences was well presented by Cooper and Crowder's demonstration of the effects of habitat structure on the process of predation. Physical structures in the habitat result in persistence of the relationship which otherwise has inherent tendency for extinction. Moreover, by filling that gap between causative explanation and resulting consequences, they were able to propose one of the few convincing prescriptions for management of ponds in terms of managing the structural features of habitat.

In a similar vein, Radovitch presents a delightful analysis of schooling, its effect on stability, and its role in destabilization as a consequence of harvesting by man. The paper is an example of adding realism to a theory, and blending that with causal understanding, population consequences, and management impact. Together with functional synthesis, it seems likely that further efforts to bridge such gaps between theory, causative explanation, and population behavior have high priority for the future.

Effects of predation exist at the community and ecosystem level as well as the population level. Predation and competition, within a given abiotic setting, do have an influence, and a dominant one, on community structure as revealed by Huntsman's analysis of reef communities. But the apparent complexity of communities has still left this area of study with weak links between explanation, consequence, and understanding. I am left applauding Larkin's plea and that of Regier, Paloheimo, and Galucci, for more analyses of ecosystems and more management of communities rather than species. But as each points out, the potential lies more in the future than the present. Analyses of ecosystem processes rather than only of population processes and the continued search for the Holy Grail of "roles" rather than species still seems to provide useful directions.

Although the book has management in the title, that is where it is weakest. There are certainly some specific examples of management notions that emerged from the functional analyses and impact descriptions. But however tactically significant such might be, the strategic framework for management was touched on by few. Dickie certainly made the point that the fisherman and his behavior are as much a part of the system as is the nonhuman predator. Not only can the fisherman, the fleet, and the fishing company be analyzed both in themselves and as they intersect with the biophysical world, but the social and economic setting for that interaction is paramount. Fisheries cannot be managed by managing fish alone. The rich and illuminating history of failures of fisheries management should make evident that people, institutions, and society are as important to investigate as are fish and water. Loftus and Byrne's description of the development of a strategy for fisheries management of lakes in Ontario provides a remarkable example of the direction to proceed. If such programs become implemented, we will see functional analyses of linked ecological, economic, and social systems, of behavioral analyses of the consequences of those linkages, leading both to better scientific understanding and better management policies. Regier et al., Dickie, and Rothschild and Forney all reinforce those arguments, emphasizing the importance of systems analytic approaches as ways to facilitate the process.

The book is a good one. It is good for its sound biological science and its sensitive use of theory and models in a realistic

framework, and it is good for highlighting what is not in the book, of the gaps in theory, in practice, and in method. There is little, for example, that provides examples of science as informed steps of simplification and generalization. Much needed attention is placed on statics and description and much less on dynamics. There is a dominant "equilibrium" and stability paradigm when much of the world is one of disequilibrium, multiequilibria, and organizational change. Fish are important but so are people, institutions, and society. Above all, the issue of the unknown, of the partially known, is touched on only in the most traditional plea for more research. The notion of management design, to produce information that separates between alternatives, is introduced by Larkin at the beginning, but does not reemerge thereafter. Those are the larger issues for the future.

C. S. HOLLING

Institute of Resource Ecology
University of British Columbia
2075 Wesbrook Mall
Vancouver, B.C.
V6T 1W5

(R-282)

Marine Benthic Dynamics. *Edited by Kenneth R. Tenore and Bruce C. Coull. University of South Carolina Press. 1980 xvii + 451 p. $27.50.*

This book is a collection of papers emanating from a symposium held at the University of South Carolina, Georgetown, South Carolina, in April 1979. Twenty-three papers are presented from benthic ecologists based at laboratories around the world.

Mills (1975) suggested that "much of benthic ecology seems a rather shabby and intellectually suspect branch of biological oceanography." Is this because many benthic scientists dwell excessively on determining structure, standing crop, taxonomy, life histories, and other seemingly dull stuff? The organizers of the symposium apparently hoped to point out that some benthic ecologists *do* think about functional relationships, equations, models, and other viewpoints that bring "vibrant ambiance" to the science. My impression from reading this book, however, is that more of the dull stuff is needed as general grist for the modeling mills. Alternatively, many benthic scientists may continue to waste their time on overly descriptive research. At least one author in the book recommends the former path: "obviously, then, one should find out as much as possible about the natural history of the species in question before predictions based on turnover ratios can be made" (Warwick, p. 18).

Many of the papers in the book deal with the changes in values of various parameters of benthic ecosystems. They document how the statistics of the attributes (biomass, nutrients, etc.) go up and down according to season or some other function. Studies of the dynamics of the meroplankton are not covered in the papers, indicating this highly relevant field is continuing to be ignored. Whether the data on population *changes* of adult benthic animals actually represent predictable *dynamics* is a moot point. Benthic ecologists obviously still lag behind planktonic ecologists in their ability to quantify and describe dynamics in a way which links organisms to milieu. This is, perhaps, because sediment dynamics are in turn lagging behind fluid dynamics and hydraulics. It is disappointing to note the profusion of correlation analyses through many of the papers. We are apparently a long way from having the luxury of predictive equations to deal with benthic populations and ecosystems.

As perhaps to be expected, the papers dealing with chemistry and nutrients are more dynamical. Nixon et al., in a comprehensive review, point out two basic problems in nutrient dynamic studies, namely, the specter of metabolic patchiness in the sediments, and the unrealistic approach of assuming simple diffusion gradients. Probably the problem arises from a lack of data on eddy diffusion coefficients near bottom, as mentioned by Propp et al. The papers on detritus present the latest results and thoughts on detritus production, utilization, and cycling. But it is curious to note that there appears to be a scarcity of information on how detritus moves through benthic ecosystems. Isn't detritus subject to water motion, and couldn't its movement be modeled? There are obvious linkages here between benthic boundary layer studies and biology. However, the intricacies between detrital food supply and population biology are largely unexplored. For example, Tenore and Rice assume that predation does not influence secondary production, and that production has a clear functional relationship with supply of detrital food.

The book is commended to scientists interested in regional benthic ecosystems, especially the northeast and northwest Atlantic, the Baltic, and the northwest Pacific. There are numerous hypotheses set forth to test, making life easier for graduate students. Students will also find the review articles comprehensive and up-to-date. Methodology and techniques are not emphasized in the papers. Applied ecologists such as fishery managers and pollution biologists would find the book only moderately useful.

The review papers are required reading for research administrators concerned with guiding marine science. These presentations show clearly that attack on certain specific questions is necessary before we can begin to predict benthic dynamics accurately and attempt to manage important and fascinating ecosystems.

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C. D. LEVINGS

Department of Fisheries and Oceans
West Vancouver Laboratory
4160 Marine Drive
West Vancouver, B.C.
V7V 1N6

(R-325)

The Biology and Management of Lobsters. Volumes 1 and 2. *Edited by J. Stanley Cobb and Bruce F. Phillips. Academic Press, Inc., New York, NY. 1980. (Vol. 1) xv + 463 p. $55.00 U.S. (Vol. 2) xiii + 390 p. $45.00 U.S.*

For many years now, lobster biologists working in North

America have been avidly awaiting the production of some volume to replace and update the old standby "Herrick" (Herrick 1896). Not since then has there been any attempt to bring together what was known between two covers, although there have been a number of bibliographies. These two volumes extend the scope of Herrick somewhat as they encompass all the species that may generally be referred to as lobsters, i.e. *Nephrops* and the rock lobsters or crayfish as well as *Homarus*. They also bring our general understanding as up-to-date as possible, given that the few months that may pass between manuscript preparation and final printing can see significant additions to knowledge.

The work was conceived at a workshop on lobster ecology held in Perth, Australia, in 1977, and the proceedings of that workshop were subsequently revised and expanded by the original contributors and several other authors to form the present two-volume work. It is organized in a sensible and straightforward manner, the first volume dealing with most aspects of physiology and behavior and the second with ecology and fisheries management.

There are, however, a number of minor irritants and inconsistencies. These, perhaps, come from the complicating factor of having some two dozen contributors attempt to cover the whole field. Thus, for example, the description that is given for the larval stages of *Homarus* is considerably less than that given for the other species, notwithstanding its simpler life history. This detail is, moveover, given in the chapter on larval ecology rather than in the chapter on general biology or in a separate chapter on morphology and anatomy where one might expect it, and I searched in vain for details of how larval *Homarus* feed, respire, excrete, and swim, and for some details of their anatomy. I was disappointed by the uneven quality of the photographs and diagrams. This is perhaps inevitable given that many illustrations had been reproduced from earlier publications. Many were excellent but others were poor and when such reproduction results in the uncertainty which pervades Fig. 4 in the chapter on Aquaculture, for example (diagram of *Homarus* hybrids), then clearly some minimum standard should be demanded. I was frustrated by the lists of contents. Each volume contains, in addition to a listing of its own contents, a list of contents for the companion volume. The idea is sound but possibly in a future edition, the second list could be put at the end where it would be less likely to be consulted in error.

It is unfortunate that some of the general descriptions should end up in specialist chapters. For example, the excellent description of management techniques problems and options for *Homarus gammarus* (Chapter 9, Vol. II) applies equally to *H. americanus*, yet the general chapter on clawed lobster fisheries lacked such detail. The casual reader might not be aware that the problems outlined so eloquently in Chapter 9 had a universality of application that the chapter title did not express.

The foregoing are perhaps carping and picayune criticisms. There is no question that a comprehensive work of this nature is long past due, and that the contributors and editors have compiled an impressive amount of information and theory, and presented it for the most part in a coherent and logical fashion. The serious student will find it an invaluable and detailed source as well as a useful introduction to the literature. It will make the task of anyone coming into lobster fisheries management or research considerably easier and should result in a major improvement in the general level of scholarship among such people. The writing, on the whole, is good and clear, although I did detect a few unexplained vernacular or local expressions. Except for a few illustrations, the books are well printed and robustly bound to withstand the hard use and long life they will undoubtedly have.

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D. J. SCARRATT

Department of Fisheries and Oceans
Atlantic Biological Station
St. Andrews, N.B.
E0G 2X0

(R-342, -343)

Marine Pollution: Functional Responses. *Edited by Winona B. Vernberg, Frederick P. Thurberg, Anthony Calabrese, and F. John Vernberg. Academic Press, Inc., New York, NY. 1979. xiii + 454 p. $23.00.*

This book contains the papers presented at a conference held late in 1977. The book is in four sections; the first three are about the effects on marine biota of petroleum hydrocarbons, metals, and pesticides and PCBs, and the fourth section is on "multiple factor interactions." Each of the first three sections begins with a review which is followed by specialized papers on various aspects of the theme.

The first section, on oil, opens with a classical review by J. W. Anderson of the fate and effects of petroleum hydrocarbons in marine biota. I thought that this provided a good summary of the current state of our knowledge of effects in terms of the biochemistry and physiology of the whole organism. Papers then follow on detoxification systems in worms, the effects of hydrocarbons on fish respiration, the effects on naphthalene on "antifreeze" protein in sculpin, amino acid composition of *Macoma* from oiled sediment, and on the sensitivity of fish larvae to oil.

Part two, on metals, begins with a review by R. Eisler of the effort devoted to research on various metals and biota from 1974 to 1976. Such a review is not as useful as Anderson's, but is interesting as a comment on what has been fashionable. During the review period, most papers were about the five or so metals which were either featured in the more notorious instances of marine pollution, or which were fairly easy to work with in observational or experimental studies. Eisler notes that there was relatively little effort directed to future possible problems of pollution. This review is followed by papers on the effects of metals on fin and limb regeneration, hematology of flounder exposed to mercury, the use of bivalves in pollution research, the effects of copper on crabs and of cadmium and copper on molluscan gills.

D. R. Nimmo opens the section on pesticides and PCBs with a review of some of the trends in the development and use of pesticides. This is useful, I think, as it suggests some of the questions which may arise during the next decade or so, and indicates the kind of research which may be useful. Then follows the now familiar mixed bag of papers dealing with the

effects of PCBs on sculpin fatty acids, parathion metabolism in invertebrates, pentachlorophenol toxicology, and a behavioral bioassay of Kepone toxicity to lugworms.

The final section of the book, on multiple-factor interactions, reflects the fact that in real life pollutants do not act as single stresses, but as parts of a variable system whose components may from time to time act synergistically or antagonistically in stressing biota. There are papers on the sensitivity of crabs to an insect growth regulator in different salinities, seasonal changes on thermal acclimation by fish, an attempt to identify the impact of salinity stress by species abundance and diversity indices, and on seasonal variations in the effects of chlorine-produced oxidants in oysters. The final chapter of the book, by C. J. Sindermann, sums up the conference. He emphasizes that at present the outstanding problem in aquatic toxicology is the extrapolation of laboratory observations of sublethal effects to impacts on populations in the field.

So much for what's in the book: how useful is it? I must reply that I do not know to whom I would recommend the book. Even though the papers and reviews may be individually satisfactory, I am not convinced that it is useful to have them published as a collection. I think that the general reader would find the reviews interesting, but the specialized papers too esoteric. The specialist may find too few papers of direct interest to him for the book to be useful. This criticism, that the book has been published without any audience clearly identified, should be directed at its editors, rather than the authors. If the real value of a scientific meeting is to help one keep abreast of developments in one's field and to exchange ideas informally (and these laudable intentions are piously stated in travel justifications), do we really need a fairly expensively produced volume of proceedings published a year after the event? I think not, and I believe that most of the contents of this book would have been more concisely produced, better refereed and edited, and more accessible, had they been published in the primary literature. This would probably have better served both author and reader.

However, the book has been published; it contains some results and some ideas which may not find their way into the primary literature, and which may be useful to someone. However, you can probably get along without the book; and if you really need a copy, you can have mine.

R. F. ADDISON

Marine Ecology Laboratory
Bedford Institute of Oceanography
P.O. Box 1006
Dartmouth, N.S.
B2Y 4A2

(R-280)

Power Plants: Effects on Fish and Shellfish Behavior. *Edited by Charles H. Hocutt, Jar R. Stauffer Jr., John E. Edinger, Lenwood W. Hall Jr., and Raymond P. Morgan II. Academic Press, Inc., New York, NY. 1980. xiii + 356 p. $25.00 U.S.*

There has been a plethora of studies over the last decade on the impact of power-generating stations on aquatic plants and animals. The editors of the present volume are themselves actively engaged in research on the effects of such power stations in the U.S. northeast. Probably because of this involvement, they have succeeded in choosing a series of topics that provide a wide sampling of problems of interest and concern to fish biologists and conscientious administrators.

Some of the chapters of the book summarize general fields of research on the subject: power plant design and fish, aggregation phenomena, the effects of temperature, water flows, biocides, entrainment of eggs and larval forms, and behavioral barriers and guidance systems. Other chapters focus on the responses of specific invertebrates to the modified environment created by the siting of power plants. There are more biologists working on fish behavior than shellfish behavior, but the text gives a balanced treatment of these ecologically and commercially important invertebrate members of the aquatic community. Blue crabs (*Callinectus sapidua*), lobsters (*Homarus americanus*), crayfish, peneid shrimp, and bivalve molluscs each have a separate chapter in which their behavior and physiology are explored.

The book also provides a balance integration of the results of carefully controlled laboratory experiments with those of field observations. These observations, while often less precise, are usually more representative of the situation existing in disturbed aquatic communities. The text is of great value to the general reader contemplating future research in any of the topics covered, since each chapter is followed by abundant literature citations (one third of the book is bibliography). The labors of the editors, chapter authors, and reviewers have produced a concise, up-to-date review of this important field of environmental biology. The book is a useful addition to the library of anyone interested in effects of power stations on aquatic organisms.

R. W. MCCAULEY

Department of Biology
Wilfred Laurier University
Waterloo, Ont.
N2L 3C5

(R-346)

Fishes of the Delaware Estuaries: a guide to the early life histories. *Johnson C. S. Wang and Ronnie J. Kernehan. EA Communications, Division of Ecological Analysts, Inc., MD. 1979. xxxi + 410 p. $22.50 U.S.*

More guides to the early development and life history of fishes have been published for the middle Atlantic coast than for any other region. There are guides for the Chesapeake Bay Region (Mansueti and Hardy 1967), the Delaware Bay (Scotton et al. 1973), the Potomac River Estuary (Lippson and Moran 1974), the mid-Atlantic Bight (Hardy et al. 1978), and now the Delaware Estuaries. It may seem that the publication of early life history guides has been "overworked" for this area, especially when similar works are desperately needed for other parts of North America. However, available knowledge on the early forms of species in this intensively studied region has been significantly updated or supplemented with each subsequent guide.

While there is much repetition among the various guides, the specific information provided and the species' coverage differ sufficiently to be considered complementary. All draw heavily upon previously published literature and supplement it with various amounts of original material to provide illustrated descriptions of eggs, larvae, and early juveniles, as well as information on adults, distribution, ecology, and reproduction. The mid-Atlantic Bight series is the most comprehensive compilation of previously published information and illustrations. Volume one of that series is essentially an updated version of the Chesapeake Bay guide. The other guides are more selective in their use of previously published data and illustrations.

The Delaware Estuaries guide includes about 69 species not covered in the Delaware Bay guide, about 42 species not covered in the Potomac River Estuary guide, and four species not covered in the mid-Atlantic Bight series. The latter four species, three lampreys and a cyprinid, are not found in any of the other guides. Conversely, both the Delaware Bay and Potomac guides include about 15 species and the mid-Atlantic Bight series about 213 species not found in the Delaware Estuaries guide.

This guide emphasizes regionally derived information (much of it original) on the early life habits and characteristics of freshwater, estuarine, and marine fishes found in the Delaware and Indian River Estuaries. When significant, differences between the authors' findings and those of others are discussed. Dichotomous keys (some of dubious value) are provided for certain developmental phases, sometimes only for the young or early juveniles, for 21 of the 50 families covered. The species' accounts consist of information on spawning location, season and temperature, salinity, and substrate; brief descriptions of early development; a discussion of various aspects of distribution, reproduction, and early life history; and lists of other pertinent references. The descriptions are limited to characters considered by the authors to be most helpful in identification. They are accompanied by selected lateral view drawings; no dorsal or ventral view illustrations are provided. Because of space limitations, there are size-related gaps in many illustrated series that could have been filled with previously published drawings. New illustrations are provided for 65 of the 112 species covered. Dorsal and anal spine and ray counts are given for most species; myomere counts are not.

The magnitude of the Delaware Estuaries guide and the time limitations in its preparation made an errorless copy almost impossible. The authors provided a two-page "errata and additions" insert which covers most of the errors.

Occasionally, a published description or illustration is based on a misidentified specimen. Such is the case for the 7.0-, 7.7-, 8.4-, and 9.7-mm TL specimens illustrated by Taber (1969) as *Micropterus salmoides* (largemouth bass). General body form, pigmentation, state of development relative to size, form of the gut, the somewhat anterior vent position, and, most dramatically, the position of the air bladder essentially posterior to, rather than above or anterior to, the coiled portion of the gut are more characteristic of certain *Lepomis* species. The specimens illustrated are probably *L. megalotis* or *L. microlophus*. Unfortunately, the authors of this guide, as well as those of the Potomac and mid-Atlantic Bight guides, have failed to recognize this error and included some or all of these drawings in their species accounts for *M. salmoides*. The smaller specimens illustrated by Taber and included in all three guides were based on material cultured from eggs collected from a largemouth bass nest (Taber personal communication) and are undoubtedly correct. Similar misidentifications may also exist elsewhere in these guides or in other published descriptions. It is imperative that they be reported in the published literature as soon as they are recognized.

There are a number of items in the introductory section under "Description of Developmental Stages" that may be disconcerting to the more experienced users of the guide. The following were particularly obvious. (1) The authors give a brief account of the sequence of events in embryonic development. Unfortunately, they do not appear to have been familiar with current theory which is effectively counter to some of the processes they described (e.g. invagination of blastoderm and formation of a hollow blastocoel, see Ballard 1973 and 1976). (2) The authors use the prolarva–postlarva terminology proposed by Hubbs (1943) rather than one of the larval phase terminologies more typically used or recommended by many of their peers (Ahlstrom 1968; Snyder 1976). (3) Although the authors do not use standard length in their species accounts, they define it here and in their glossary, and illustrated it in Fig. B, as "the distance from the tip of the snout to the point of inflexion of the urostyle" rather than to the more conventional posterior-most margin of the hypural bones or plate(s). (4) The authors fail to provide criteria for differentiating preanal and postanal myomeres above the posterior margin of the vent. The Siefert (1969) method, which includes all myomeres transected by an imaginary vertical line from the posterior margin of the vent as part of the preanal count, has been accepted by many of the authors' peers.

The faults of the guide are far overshadowed by its contributions to our knowledge of the early development and life history of the many species covered. While this information is regionally oriented or derived, the guide should prove useful to many aquatic biologists throughout North America.

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D. E. SNYDER

Department of Fishery and Wildlife Biology
Larval Fish Laboratory
Colorado State University
Fort Collins, CO 80523, USA (R-268)

Evolution and Ecology of Zooplankton Communities. *Special Symposium Volume 3. Edited by W. Charles Kerfoot. University Press of New Enland, Hanover, NH. 1980. xxiii + 793 p. $45.00 U.S.*

This volume consists of the text of almost all the papers presented at a symposium on zooplankton ecology held at Dartmouth College, Hanover, NH, in August 1978. Its purpose, to paraphrase the editorial preface, was to provide opportunities for synthesis and creative thought in areas of aquatic ecology that have recently received a great deal of research interest and theoretical development. The chosen format of the symposium was a carefully organized program with frequent formal discussion periods, and this was closely followed in the printed proceedings. As stated in the preface, the approach was to develop a picture of community structure based on a firm base of individual physiology and behavior integrated at the population level by the mechanisms of competition and predation.

Taken as a whole an overview of the papers presented and their arrangement suggests a coherent pattern in zooplankton research at least in the minds of the organizers. The volume starts with a paper dealing with the consequences to small organisms of living in a viscous environment. Several of these consequences are explored further in the following sections which make up roughly the first half of the book. Papers in the first five sections deal primarily with individual morphology, responses and physiology related to problems of search for and recognization of mates (or prey), avoidance of predators, feeding and causes of horizontal and vertical distributions, and migrations.

A section on the interaction of algal communities and zooplankton introduces what I feel is the theme of most of the rest of the book. The physiological ecology approach of the first sections sets the stage for and develops the theme of selective grazing by zooplankton as a force, if not the only force, shaping algal communities. It requires little effort then to turn to the other aspect of the Brooks and Dodson (1965) hypothesis of the control of aquatic communities, namely the size-selective grazing of zooplankton by fish and other predators which appears to be the major subject for the second half of the volume.

The sequence in this part shows the same degree of control as in the first. Individual and population characteristics are dealt with first, then the resultant supporting evidence for selective predation is documented, and finally community and system consequences are considered. The first section of this half of the volume deals with reviews of the genetics, population dynamics, and reproductive strategies in freshwater zooplankton groups while the following one deals with cyclomorphosis, which editor Kerfoot strongly believes is a necessary adaptive strategy to minimize the effects of predation in the predominately parthenogenic rotifers and cladocerans with short life cycles and direct development. The next section describes the effects of predation on zooplankton populations by a wide range of invertebrate and vertebrate predators. The last two sections attempt to round out the size-selective predation picture with articles of a rather steady-state nature, community structure and spatial distribution patterns, and finally several more dynamic interspecies interactive studies and models. The overall organization of the volume rather than the subject matter for any one paper appears to be the framework against which the title for the volume was chosen.

Despite the emphasis on freshwater zooplankton, especially cladocerans and copepods, there is a seven page double-column taxonomic index indicating a wide range of references to diverse zooplankton organisms, and several papers refer primarily to rotifers, a few to *Chaoborus*, and one or two to water mites. Much of the grazing section describes work on marine calanoid copepods. In addition to the range of taxonomic material, there is a wide range of treatment in the 66 papers included. The range is from very descriptive through the whole gamut of analytical, theoretical, and review papers. Some are very vague. For example, the running commentary describing methods for high-speed photomicrography of particle selection by a calanoid is fine stuff for an oral presentation, but not much help for someone trying to repeat the technique! A paper on the viscous environment concentrates too much on the derivation of Reynolds and Strouhal numbers and too little on their consequences. I found many of the case history papers clear, concise, and interesting; most of the analytical analyses and reviews present a lot of data and develop interesting, often controversial, points of view. In general, conference organizers and editor have successfully fulfilled their aims of putting together a cohesive view of zooplankton ecology emphasizing the importance of environmental limitations and individual behavioral responses.

What of the symposium aims of stimulation of "creative ferment, synthesis and lively debate?" We read that the prepared papers were to be liberally dispersed with ample time for discussion, yet there is little report of what passed in those sessions. One can sense, as one reads the volume, differences of opinion among the authors even in the highly selected format. Gliwicz, for instance, allows selective grazing as a competitive factor between large and small cladocerans, but feels that changes in algal species with eutrophication affect changes in cladoceran dominance rather than vice versa. Kerfoot discounts cyclomorphosis in copepods while Einsle presents the puzzling case of *Cyclops abyssorum* in Italian

montaine lakes. Confer et al. contend that cladoceran eye diameter changes diurnally as a form of accommodation to light and dark; this is rejected by Kerfoot. What is the status of cladoceran morphs? Are they genetically distinct, successive populations or are they selected by seasonal changes in predation? How does diapause fit into this? Certainly these issues and many more raised in the pages of prepared text are the meat for much discussion, synthesis, and future research. Did any of this ferment take place in the roughly 10 h of scheduled discussion during that late August week of 1978? If so, it is a pity that more of it did not reach the readers of this volume who were not fortunate enough to attend. I feel that there certainly would have been room in the volume if some of the material which has already been published elsewhere had been eliminated for reporter's summaries of discussion groups.

I think this attractive volume, produced in the format of the sponsoring organization, American Society of Limnology & Oceanography (ASLO), will be widely used for the quantity of documentation of zooplankton interactions with phytoplankton on one hand, and with predators, both invertebrate and vertebrate, on the other, as well as for some of the excellent reviews, synthesis, and theoretical formulations it contains. It is to be regretted that its well thought-out format could not fully represent the dynamic ferment it hoped to create.

BROOKS, J. L., AND S. I. DODSON. 1965. Predation, body size and composition of zooplankton. Science 150: 28–35.

N. H. F. WATSON

Ocean Science and Surveys
Central Region
Department of Fisheries and Oceans
Canada Centre for Inland Waters
P.O. Box 5050
Burlington, Ont.
L7R 4A6

(R-348)

Parasitic Copepoda of British Fishes. *(Ray Society Publication No. 152). Z. Kabata. British Museum (Natural History), England. 1979. vi + 468 p. (2031 Figures). £ 30.*

Kabata's monograph is very much more than a compendium of the parasitic copepods of fish found around the British Isles. It begins with a comprehensive revision of the systematics of both free-living and parasitic copepods. Seven orders are recognized: Harpacticoida, Misophrioida, Monstrilloida, Calanoida, Cyclopoida, Poecilostomatoida, and Siphonostomatoida. A plausable scheme for the evolution of these orders from a benthic ancestor is proposed, based on patterns of body tagmosis and the morphologies of oral appendages.

For the next 400 pages, and with 67 text figures and 2031 plate figures, the systematics of 20 families (1, Cyclopoida; 5, Poecilostomatoida; and 14, Siphonostomatoida) are expored. Each family is morphologically analyzed for phylogenetic trends. A taxonomic history of each family is given Member genera are made morphologically and ecologically intelligible. A detailed diagnosis of each species found in the British Isles is given. Keys are provided to aid in identifying all families, genera, and species. Host–parasite relations are explored as much as possible.

It will no longer be possible to study the nomenclature, systematics, or ecology of parastic copepods without this book. It will no longer be possible to opine on general copepod evolution without knowledge of this book. These declarations are unequivocal.

Two trivial complaints are possible. Figures might have been identified directly on the plates. Figure legends in the text might have been lengthened so that figure and legend could stand alone without textual reference.

Two substantive criticisms can be raised at this point. First, Kabata divides the Copepoda, as many others have before him, into the Gymnoplea (the calanoids) and Podoplea (all other orders). This distinction is based upon the position of the articulation between the wide anterior end (prosome) and narrow posterior end (urosome) of a copepod's body. This character in the Gymnoplea is probably apomorphic and the group represents a monophyletic assemblage. Two views of the Podoplea are possible. First, the character that distinguishes them may be regarded as plesiomorphic and the group is probably polyphyletic and should not be recognized by a single taxonomic name. Second, the position of the prosome–urosome articulation in the podopleans can be regarded as apomorphic and the Gymnoplea and Podoplea may have been derived from a common ancestor (Kabata's hypothesis). However, no copepod, extant or fossil, has been found with the requisite combination of characters to substantiate this latter hypothesis. Thus, the division of the Copepoda retained by Kabata should be regarded as tentative.

A second criticism which must be voiced concerns Kabata's order Siphonostomatoida. It includes all copepods with mouth parts formed into a buccal cone or tube containing mandibular stylets. This assemblage can be divided into those with cyclopoid-like bodies and appendages which are parasites of invertebrates (not included in Kabata's monograph) and those parasites of vertebrates with more or less highly modified morphologies. Details of the oral morphology of these two groups differ enough for convergence to be suspected (as Kabata notes). If convergence is assumed, the order can be regarded as at least diphyletic, and a single ordinal designation is not warranted. Resolution of these difficulties awaits a systematic study of the parasitic copepods of invertebrates, which was not Kabata's subject.

These criticisms noted, the reader must recognize two characteristics of Kabata's work. First, the evolutionary topography of copepod diversity and especially that of parasitic copepods is so unexplored that any study of it remains pioneering and provisional. Later generations will probably recognize that Kabata took some wrong or dead-ending turns, following, as he had to, his own landmarks and those of the very few brave explorers before him. Once the terrain has been better mapped (especially among the siphonostomatoids), it will be easy for followers to see a clearer route. This fact enhances the importance of the present work; it can never diminish it. In this respect, Kabata joins the ranks of other legendary copepodologists such as Baird, Brady, Giesbrecht, Claus, Sars, T. and A. Scott, Wilson, Gurney, and Lang.

Second, aside from the rational question of whether Kabata's ordinal and familial phylogenetic hypotheses are true, his monograph demands evaluation, a question of quality. It is good? Paraphrasing Lonergan, the good is always concrete; it is never abstract. Definitions are abstract, therefore one cannot define the good. The best one can do is show an enquirer a good painting, a good piece of sculpture, a good opera, or a good person. Kabata has provided his intellectual heirs — aspiring novices and seasoned professionals alike — a paradigm of good scholarship. Kabata and the Ray Society have done the present world and the next century a great deal of good with this beautiful, comprehensive, and compelling monograph.

B. M. MARCOTTE

McGill University
Institute of Oceanography
3620 University Street
Montreal, Que.
H3A 2B2

(R-308)

Ecology of Freshwaters. *Brian Moss. Halsted Press (Division of John Wiley & Sons), New York, NY. 1980. xv + 332 p. $24.95 U.S.*

This is an attractive paperback book that is sturdy enough to stand considerable abuse. There is much about it that I like. It is written in fluent, readable English. It considers very recent literature in a synthetic and critical fashion. It has a high enough content of Canadian science to make it the textbook of choice for most basic aquatic biology courses in this country, and yet it is cosmopolitan in its treatment (a critical consideration when dealing with students whose concepts of Far East and Deep South are Toronto and Minneapolis). One gets the feeling that the author knows all of the ecosystems he discusses firsthand, whether Arctic or tropical. There are a few bloopers which will tip off limnologists native to particular areas that Moss is not a long-term resident of their biome, but only a well-trained limnological spy. For example, in Chapter 2 the fact that "freezeout" of oxygen from ice cover may totally overwhelm decomposition in its effect on oxygen concentrations in northern latitudes is not discussed. The statement that plankton concentrations are much greater in the epilmnion than in the hypolimnion does not generally hold (Fee 1976 and references therein).

The book has good chapters on topics which other general texts treat superficially, if at all (e.g. nekton, streams, macrophytes, and paleoecology). In contrast to chemical and plankton chapters, the one on nekton largely ignores North American fishes (but it is presumably not written primarily for North American students in any case). The chapter on paleoecology skims somewhat superficially over problems with recent dating, particularly with bomb cesium. Best of all, I liked the lead-off chapter which firmly establishes the relationship of a lake and its watershed, a non-Forbesean stance which I wholeheartedly endorse. I would have liked to see one further step, i.e. the inclusion of a lake's airshed. There is little on the exchanges between water and atmosphere which are critical in the cycling of some nutrients and toxins. Also, more on the physical dynamics that make the thermocline an important barrier to nutrient exchanges would have been welcome. It seems that some chemical sections would have given more balance to the stream chapter, perhaps a bit more on Hubbard Brook.

In a nutshell, more of chemistry and physics would have balanced the biology. These are only minor complaints, and few recent scientific books exhibit such fine craftsmanship. Brian Moss deserves congratulations for a job well done. This book will be useful for many years to come.

FEE, E. J. 1976. The vertical and seasonal distribution of chlorophyll in lakes of the Experimental Lakes Area, northwestern Ontario: implications for primary production estimates. Limnol. Oceanogr. 21(6): 767–783.

D. W. SCHINDLER

Department of Fisheries and Oceans
Western Region
Freshwater Institute
501 University Crescent
Winnipeg, Man. R3T 2N6

(R-327)

Books received/Livres reçus[1]

March/mars 1981

***The Computation of Dissolved Gas Levels as a Function of Temperature, Salinity, and Pressure.** *John Colt. University of California, Davis, CA. 1980. vi + 81 p.* (This report serves as a convenient source of information on the solubility of oxygen, nitrogen, and argon under a variety of conditions. The oxygen solubility data is also presented on a one-page table that can be placed on the wall. Both reports are free while the supply lasts and can be obtained from: *John Colt, Department of Civil Engineering, University of California, Davis, CA 95616, USA.*)

***Common Intertidal Invertebrates of the Gulf of California, Second Edition.** *Richard C. Brusca. University of Arizona Press, Tucson, AZ. 1980. 513 p. $26.95 U.S.* (This second edition of the handbook, which discusses some 1300 species of marine invertebrates, as well as a few plants and fish, presents the first scheme of intertidal zonation for the Gulf of California.)

***Resource Management and Environmental Uncertainty: Lessons from Coastal Upwelling Fisheries.** *Edited by Michael H. Glantz and J. Dana Thompson. John Wiley & Sons, Toronto, Canada. 1981. xvii + 491 p. $42.50 U.S.* (Dealing primarily with resource management in marine upwelling areas, a number of contributors cover both the physical–biological and political problems involved.)

April/avril 1981

***Aquatic Microbiology.** *G. Rheinheimer. John Wiley & Sons, New York. 1980. 235 p. $28.00 U.S.* (This is the second English edition of *Aquatic Microbiology*. It still remains an introduction to aquatic microbiology, albeit extended over the first edition, aimed at providing a survey of the present position of this branch of study for readers from science, teaching, and practice.)

***Fisheries Biology: A Study in Population Dynamics (2nd ed.)** *D. H. Cushing. The University of Wisconsin Press. 1981. xvi + 295 p. $17.00 U.S.* (This new edition, completely revised, takes into account the considerable amount of research conducted since publication of the original edition. Dr Cushing has rearranged the material, pointing it to graduate students and practicing fisheries biologists.)

***Rethinking Artisanal Fisheries Development: Western Concepts, Asian Experiences.** *Donald K. Emmerson. The World Bank, Washington. 1980. x + 97 p. Free.*

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[1]Entries in this list *not* marked by an asterisk are available for review by interested readers for a 3-month period from the time of publication of the list. For information, please write to the book Review Editor.

[1]Les ouvrages inclus dans cette liste et *non* accompagnés d'un astérisque peuvent être mis à la disposition de lecteurs intéressés qui voudraient en faire l'analyse, et ce, pour une période de 3 mois à compter de la date de parution de la liste. Pour plus de renseignements, veuillez écrire au rédacteur des Comptes rendus.

ANNOUNCEMENTS/AVIS

Symposium on the Acquisition and Utilization of Aquatic Habitat Inventory Information

The Western Division of the American Fisheries Society is organizing this symposium to be held in Portland, Oregon, from October 28–30, 1981. Participation in the symposium by anyone interested in aquatic habitat inventories is encouraged.

Topics for discussion at the symposium will include the following: Defining the aquatic ecosystem and its individual components; Methodology for measuring the habitat components; Relating habitat components to species utilization; Interfacing aquatic habitat inventories with other resource inventories; Using habitat inventory information to evaluate habitat condition and potential; The use of inventory information and evaluation procedures in aquatic habitat classification systems; The use of inventory data and evaluations in resource planning and impact analysis; Data storage; retrieval, and analysis systems. For more information about the program or for information on purchasing proceedings, please contact: *Neil B. Armantrout, General Chairman, Aquatic Habitat Symposium, P.O. Box 2965, Portland, OR 97208. Telephone no. (503) 231-6870.*

Colloque sur l'acquisition et l'utilisation des données d'inventaires d'habitats aquatiques

La division ouest de l'*American Fisheries Society* organise ce colloque qui se tiendra à Portland (Orégon) du 28 au 30 octobre 1981. Quiconque est intéressé aux inventaires des habitats aquatiques est invité à y participer.

Parmi les sujets de discussion, on note les suivants : Définition de l'écosystème aquatique et de ses composants; Méthodologie de la mesure des composants d'un habitat; Relations entre composants de l'habitat et utilisation des espèces; Parallèles entre inventaires d'habitats aquatiques et ceux d'autres ressources; Utilisation des données d'inventaires d'habitats dans l'évaluation des conditions et du potentiel de l'habitat; Utilisation des données d'inventaires et des méthodes d'évaluation dans les systèmes de classification d'habitats; Utilisation des données d'inventaires et d'évaluations dans la planification des ressources et les analyses d'impact; Banques de données, systèmes de recherche et d'analyse. Pour de plus amples renseignements sur le programme et sur l'achat des comptes rendus, on est prié de communiquer avec: *Neil B. Armantrout, General Chairman, Aquatic Habitat Symposium, P.O. Box 2965, Portland, OR 97208. No. de téléphone (503) 231-6870.*

Call for Papers — Spruce Budworm Conference

The Eastern Spruce Budworm Research Conference will be held at the University of Maine, Orono, ME, during the week of January 4, 1982. Papers and posters are invited. Preliminary abstracts must be received no later than September 1, 1981, and should be submitted to: *Mr Philip J. Malerba, Special Projects Forester, St. Regis Paper Company, Northern Timberlands Division, Bucksport, ME 04416. Telephone no. (207) 469-3131 Ext. 431.*

Appel d'articles — Conférence sur la tordeuse des bourgeons de l'épinette

La *Eastern Spruce Budworm Research Conference* se tiendra à l'Université du Maine, à Orono (Maine), dans la semaine du 4 janvier 1982. On est invité à présenter des articles et des affiches. Des résumés préliminaires devront être soumis, avant le 1er september 1981, à: *M Philip J. Malerba, Special Projects Forester, St. Regis Paper Company, Northern Timberlands Division, Bucksport, ME 04416. No. de téléphone (207) 409-3131 poste 431.*

International Symposium — Fish Reproduction: tactics and strategies

The Fisheries Society of the British Isles will hold an international symposium on *Fish Reproduction: tactics and strategies* at Plymouth Polytechnic, Plymouth, Devon, U.K., July 19–23, 1982. Closing date for bookings will be March 31, 1982.

Sessions will include the following: Reproductive strategies of taxonomically related groups: Reproductive strategies in fish communities; Reproductive strategies — implications for exploitation; Unusual reproductive strategies; Physiological tactics in reproduction; Behavioural tactics in reproduction. Further details can be obtained from *Dr R. J. Wootton, Department of Zoology, University College of Wales, Aberystwyth SY23 3DA, Dyfed, Wales, U.K.*

Colloque international — Reproduction des poissons: tactiques et stratégies

La *Fisheries Society of the British Isles* organise un colloque international sur la *Reproduction des poissons: tactiques et stratégies*, qui se tiendra au Plymouth Polytechnic, Plymouth, Devon (Royaume-Uni), du 19 au 23 juillet 1982. La date limite pour les entrées a été fixée au 31 mars 1982.

Les séances porteront sur les sujets suivants : Stratégies de reproduction chez des groupes taxonomiquement apparentés; Stratégies de reproduction parmi les communautés de poissons; Stratégies de reproduction — implications pour l'exploitation; Stratégies de reproduction exceptionnelles; Tactiques physiologiques de reproduction; Tactiques de comportement dans la reproduction. Pour plus de détails, on est prié de s'adresser au *Dr R. J. Wootton, Department of Zoology, University College of Wales, Aberystwyth SY23 3DA, Dyfed, Wales (U.K.).*

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Ammonia volatilization in a hypertrophic prairie lake
T. P. Murphy and B. G. Brownlee

Blue-green algal ammonia uptake in hypertrophic prairie lakes
T. P. Murphy and B. G. Brownlee

Screen mesh size affects estimates of macro- and meio-benthos abundance and biomass in the Great Lakes
T. F. Nalepa and A. Robertson

Variations in the correlation of body size with concentrations of Cu and Ag in the bivalve *Macoma balthica*
C. R. Strong and S. N. Luoma

The pathogenicity of *Vibrio anguillarum* for juvenile winter flounder, *Pseudopleuronectes americanus*
W. D. Watkins, R. E. Wolke, and V. J. Cabelli

Remote acoustic detection of zooplankton response to fluid processes, oceanographic instrumentation, and predators
M. H. Orr

Methods for estimating secondary production in marine Amphipoda
D. J. Wildish and D. Peer

Specific growth rates of freshwater algae in relation to cell size and light intensity
D. A. Schlesinger, L. A. Molot, and B. J. Shuter

Form of random variation in salmon smolt-to-adult relations and its influence on production estimates
R. M. Peterman

Demography of an estuarine amphipod (*Gammarus lawrencianus*) experimentally selected for high "r": a model of the genetic effects of environmental change
R. W. Doyle and W. Hunte

The variability of line intercept density estimates (a simulation study of the variance of hydroacoustic biomass estimates
D. K. Kimura and N. A. Lemberg

Plasma corticosteroid dynamics in channel catfish (*Ictalurus punctatus*) exposed to ammonia and nitrite
J. R. Tomasso, K. B. Davis, and B. A. Simco

The bias and loss of precision due to tag loss in Jolly–Seber estimates for mark–recapture experiments
A. N. Arnason and K. H. Mills

A versatile growth model with statistically stable parameters
J. Schnute

Vertical and horizontal distribution patterns of copepods near the shelf break south of Nova Scotia
A. W. Herman, D. D. Sameoto, and A. R. Longhurst

Salt and heat budgets for the Gulf of St. Lawrence
G. L. Bugden

Notes

Marking fish by scarring soft fin rays
H. E. Welch and K. H. Mills

Book reviews/Comptes rendus: Aquatic oligochaete biology (R. O. Brinkhurst and D. G. Cook [ed.]) **K. Stimpson**; Epithelial transport in the lower invertebrates (B. Lahlou [ed.]) **D. Randall**; Predator–prey systems in fisheries management (H. Clepper [ed.]) **C. S. Holling**; Marine benthic dynamics (K. R. Tenore and B. C. Coull [ed.]) **C. D. Levings**; The biology and management of lobsters. Volumes 1 and 2 (J. S. Cobb and B. F. Phillips [ed.]) **D. J. Scarratt**; Marine pollution: functional responses (W. B. Vernberg, F. P. Thurberg, A. Calabrese, and F. J. Vernberg [ed.]) **R. F. Addison**; Power plants: effects on fish and shellfish behavior (C. H. Hocutt, J. R. Stauffer Jr., J. E. Edinger, L. W. Hall Jr., and R. P. Morgan II [ed.]) **R. W. McCauley**; Fishes of the Delaware Estuaries: a guide to the early life histories (J. C. S. Wang and R. J. Kernehan) **D. E. Snyder**; Evolution and ecology of zooplankton communities (W. C. Kerfoot [ed.]) **N. H. F. Watson**; Parasitic Copepoda of British fishes (Z. Kabata) **B. M. Marcotte**; Ecology of freshwaters (B. Moss) **D. W. Schindler** 1006–1015

Books received/Livres reçus 1016

Announcements/Avis 1017

Coming next month/À paraître le mois prochain 1018

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CONTENTS

1. Planning the Article
2. Writing the Article
3. The Final Draft
4. Editorial Review of Manuscripts
5. Manuscript into Print
6. Proof Correction
7. Indexing
8. General Style Conventions
9. Style in Special Fields
10. Abbreviations and Symbols
11. Word Usage
12. Annotated Bibliography

Index (25 pages)

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CONTENTS

1. Clearing Away the Underbrush
2. The Ground Plan
3. The Master Plan
4. The First Draft
5. The First Revision: Structural Alterations
6. Further Revision: Polishing the Style
7. Editing Assignments
8. The Final Steps
9. Responding to the Editor
10. Designs of Tables and Figures
11. Preparation for Writing the Doctoral Thesis
12. Writing a Research Project Proposal
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ISSN 0706-652X

Volume 38, No. 8, August 1981 Volume 38, n° 8, août 1981

Numerical classification of sharp-beaked redfishes, *Sebastes mentella* and *S. fasciatus*, from northeastern Grand Bank **I. H. Ni** 873–879

Acclimation to copper by rainbow trout (*Salmo gairdneri*) — a modifying factor in toxicity **D. G. Dixon and J. B. Sprague** 880–888

Abiotic versus biotic uptake of radiophosphorus in lake water **S. J. Tarapchak, D. R. Slavens, and L. M. Maloney** 889–895

Patterns of invertebrate size and abundance in aquatic habitats **G. G. Mittelbach** 896–904

Culture à dialyse : emploi de fibres creuses dialysantes pour la culture massive de phytoplancton **P. Marsot, R. Fournier et C. Blais** 905–911

Effect of the algal length/aperture length ratio on Coulter analyses of lake seston **H. A. Vanderploeg** 912–916

Uptake of cadmium in perfused rainbow trout (*Salmo gairdneri*) gills **P. Pärt and O. Svanberg** 917–924

Establishment of walking catfish (*Clarias batrachus*) cell lines and development of a channel catfish (*Ictalurus punctatus*) virus vaccine **E. J. Noga and J. X. Hartman** 925–930

Fate of Kepone and mirex in a model aquatic environment: sediment, fish, and diet **D. R. Skaar, B. T. Johnson, J. R. Jones, and J. N. Huckins** 931–938

Introgressive hybridization in populations of Paiute cutthroat trout (*Salmo clarki seleniris*) **C. A. Busack and G. A. E. Gall** 939–951

Determination of mineral fiber concentrations in fish tissues **A. R. Batterman and P. M. Cook** 952–959

Relationships between nannoplankton and lake trophic status **S. Watson and J. Kalff** 960–967

Reproductive uncertainty and the evolution of iteroparity: why don't flatfish put all their eggs in one basket? **D. A. Roff** 968–977

Notes

Release of phosphorus by certain benthic invertebrates **W. S. Gardner, T. F. Nalepa, M. A. Quigley, and J. M. Malczyk** 978–981

A method for comparing the precision of a set of age determinations **R. J. Beamish and D. A. Fournier** 982–983

Increased toxicity of ammonia to rainbow trout (*Salmo gairdneri*) resulting from reduced concentrations of dissolved oxygen **R. V. Thurston, G. R. Phillips, R. C. Russo, and S. M. Hinkins** 983–988

Abundance, life history, and growth of introduced populations of the opossum shrimp (*Mysis relicta*) in subalpine California lakes **M. D. Morgan** 989–993

Absorption of selected antimicrobic drugs from water by channel catfish, *Ictalurus punctatus* **K. E. Nusbaum and E. B. Shoots Jr.** 993–996

Absence of the protozoan *Myxosoma cerebralis* (Myxozoa: Myxosporea), the cause of whirling disease, in a survey of salmonids from British Columbia **L. Margolis, T. E. McDonald, and G. E. Hoskins** 996–998

Determination of free metal ion concentrations using bioassays **U. Borgmann** 999–1002

On estimating partial recruitment in virtual population analysis **D. A. Roff** 1003–1005

(*Continued inside back*)